Food Research

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A STUDY OF THE EFFECTS OF CERTAIN FOOD EMULSIFIERS IN HAMSTERS [a]

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Department of Physiological Chemistry, Wayne State University College of Medicine, Detroit

(Manuscript received March 14, 1957)

During recent years a number of compounds, known as non-ionic emulsifiers, have been tested and some used as additives to bread and other baked goods to improve texture and to assist in the retention of softness and taste. The amount used for this purpose is usually less than 0.5%. It is necessary, of course, in each case, to establish the safety of the additive by appropriate studies in experimental animals. The present investigation deals with the effects of two emulsifiers, glyceryl-monostearate [b] and polyoxyethylene-8-stearate (Myrj®45 [c]), when fed to hamsters given a basal purified diet for periods of 22 to 28 weeks. The basal purified diet was found (unpublished data) to support excellent growth in young hamsters, comparable with that obtained from a "natural" diet. During the experiment, the growth, food intake, and gross appearance of the animals were carefully followed. At the end of the experimental period, each animal was autopsied and examined for any gross abnormality, such as variations in organ size, tumors or abnormal growths, infections, and calculi. Sections of liver, kidney, bladder, and testis from a number of animals were taken for histopathological examination.[d] Animals succumbing during the experiment were likewise autopsied and, unless extensive postmortem changes had occurred, tissues were taken for microscopic examination. Two series of hamsters were studied in two consecutive years, 1954–55 and 1955–56.

EXPERIMENTAL AND RESULTS

First Series. Sixty weanling male hamsters ("Golden Syrian") were fed a purified diet consisting of the following:

24% casein	3% Cellu-flour
52% sucrose	1% dried brewer's yeast
15% corn oil	1% cod liver oil
4% Wesson salts	

An adequate synthetic vitamin mixture was included in a portion of the sucrose.

After a 3-week "equilibration" period, hamsters were divided into 4 groups, 15 animals each, and were fed a "control" diet or diets containing 5 or 15% levels of Myrj 45 or 5% glyceryl monostearate. Appropriate corrections for the caloric and inert residue were

[a] Aided by a grant from the Atlas Powder Company, Wilmington, Delaware.

[b] The concentrated glyceryl monostearate used in this study was designated Myverol® 18–00, control No. 9017, manufactured by Distillation Products Industries, Division of Eastman Kodak Company, Rochester, New York.

[c] The Myrj®45 used in this study was supplied by the Atlas Powder Company, Wilmington, Delaware.

[d] We are indebted to Dr. T. Hiratzka, Department of Pathology, Wayne State University College of Medicine, for the histopathological examination of the tissues herein reported.

TABLE 1

Composition of diets of various groups

Diet	Group				
	1	2	3	4	5
	Control	5% Myrj 45	15% Myrj 45	5% Glyceryl Mono-stearate	15% Glyceryl Mono-stearate
Casein	24	24	24	24	24
Sucrose	42	42	42	40.7	38.3
Vitaminized sucrose[1]	10	10	10	10	10
Corn oil	15	13.6	10.8	12.7	8.1
Cod liver oil	1	1	1	1	1
Wesson salts[2]	4	4	4	4	4
Celluflour[3]	3	3	3	3	3
Dried brewer's yeast	1	1	1	1	1
Agar	3.6	----	----	2.2	6.3
Myrj 45	----	5	15	----	----
Glyceryl monostearate	----	----	----	5	15

Figures for groups 1, 2 and 4 each total 103.6% due to 3.6% "inert" material in each diet. The total for groups 3 and 5 is 110.8%, respectively, due to larger amount of "inert" constituents.

[1] *Vitamin Mixture* (amounts/1000 g. mixture): Thiamine, 100 mgm., Riboflavin, 100 mgm., Pyridoxine, 100 mgm., Niacin, 400 mgm., Pantothenic Acid, 400 mgm., Inositol, 10 g., Folic Acid, 20 mgm., Vitamin B_{12}, 10 microgm., Biotin, 10 mgm., PABA, 3 g., Choline (Cl), 20 g., Vitamin K, 300 mgm., Sucrose, 965 g.

[2] Wesson, L. G., *Science*, **75**, 339 (1932).

[3] Cellu-flour from The Chicago Dietetic Supply House Inc., Chicago, Ill.

made. Exact compositions of the diets are given in Table 1. Water was supplied *ad libitum*. Laboratory temperature was maintained at 76–78° F. All possible cleanliness was maintained in the cages and food receptacles. The 5% glyceryl monostearate group was sub-divided after 8 weeks. Five were continued and 10 were changed to the 15% level. Body weights of all animals were taken weekly, food consumption data recorded, and the animals observed carefully for any gross symptoms. Any animals succumbing were autopsied and sections of tissue taken for histological examination. At the end of the 28-week period, the remaining animals were autopsied and sections of liver, kidney, bladder, and testis were taken for histopathologic examination.

RESULTS

Average body weights of the 5 groups of animals are given in Table 2. It is evident that there is no definite difference in the body weights of the various groups of hamsters. This is borne out by the statistical analysis of the group average body weight data (Table 3). It is evident that since "P" far exceeds 0.05, when the 4 groups are compared with the corresponding glyceryl monostearate groups, there is no significant difference between average weights of the various groups either after 10 weeks or 22 weeks on the experiment. It is interesting that the weights of the animals given 15% Myrj 45 are similar to those of the other groups despite the persistent diarrhea and the death of several of the group due to respiratory infection. The slight decline in body weight noted at the 26th week (Table 2) was due to a temporary decrease in food intake as a result of a hot period of weather during which the control of the animal room temperature was inadequate. A subsequent return of the temperature to the desired 76–78° range was followed by a prompt rise in food intake and body weight.

A statistical analysis of the gains in weight of the various groups over the first 10 weeks, the period of active growth, is given in Table 4. The "P" value has been calculated by two entirely different procedures for the purpose of an additional cross-check of significance. Since the P value exceeds 0.05 in every case, it is evident that there is no significant difference between the gains in weight over the growth period between any of the groups.

TABLE 2

Average body weight of various groups

	Number of animals	Week									
		0	4	8	12	16	20	22	24	26	28
Control	15	86	111	126	126	125	127	126	124	121	124
5% Myrj 45	15	86	113	132	133	127	119	121	120	118	122
15% Myrj 45	15	86	109	126[1]	127	128[2]	128	127	129[3]	125	126
5% Glyceryl Monostearate	5	86	113	124	132	130	129	128	127	126	128
15% Glyceryl Monostearate	10	....	.....	133	130	129	121	121	121	120	124

[1] 9 survivors.
[2] 8 survivors.
[3] 7 survivors.

TABLE 3

Statistical analysis of group average weights of various groups

		Control	5% Myrj 45	15% Myrj 45	5% Glyceryl Mono-stearate	15% Glyceryl Mono-stearate
10 weeks	Number of animals	15	15	9	5	10
	Average weight (g.)	130	135	129	130	136
	Standard deviation	±11.6	±15.2	±17.6	±21.7	±16.1
	"t" Value[1]	.....	0.309	0.051	0.000	0.328
					0.171[2]	0.272[2]
	P[1]	.....	>0.5	>0.5	>0.5	>0.5
					>0.5[2]	>0.5[2]
22 weeks	Number of animals	15	15	8	5	10
	Average weight (g.)	126	121	127	128	121
	Standard deviation	±10.2	±10.9	±21.8	±22.7	±22.5
	"t" Value[1]	.....	0.395	0.045	0.081	0.229
					0.275[2]	0.541[2]
	P[1]	.....	>0.5	>0.5	>0.5	>0.5
					>0.5[2]	>0.5[2]

[1] Compared with control group. For calculation, see Snedecor, *(10)*, pp. 65 and 81.
[2] Compared with appropriate Myrj 45 group.

Average daily food intake of the various groups is given in Table 5. Daily protein intake and also gain in body weight both per gram of food and per gram of protein were calculated. No marked difference between values of the various groups is evident unless possibly there is a better utilization of food in the groups given 5% Myrj 45 or 5% glyceryl monostearate. Further observations on this point were made in the second series of animals, discussed subsequently.

During the experiment, 8 hamsters died, all in the 15% level Myrj 45 group. Six died during the 6th week, one during the 15th week, and one during the 23rd week. Autopsy revealed no renal or bladder calculi or other abnormalities other than hemorrhagic congestion of the lungs. Severe diarrhea was evident in each case. Death was

TABLE 4

Statistical analysis of gains in weight of various groups over the ten-week period of growth

	Control	5% Myrj 45	15% Myrj 45	5% Glyceryl Mono-stearate	15% Glyceryl Mono-stearate
Number of animals	15	15	9	5	10
Average weight gain (g.)	43	48	45	45	46
Actual range (g.)	20-54	30-62	33-63	28-58	36-65
Standard deviation (g.)	±8.9	±9.0	±8.7	±10.7	±8.6
"t" Value[1]	.....	1.49	0.167	0.124	0.254
				0.184[2]	0.075[2]
P[1]	.....	0.154	>0.5	>0.5	>0.5
				>0.5[2]	>0.5[2]
P[3]	.....	0.097	>0.5	0.46	0.989
				0.879[2]	0.974[2]

[1] Compared with control group. For calculation, see Snedecor, *(10)*, pp. 65 and 81.
[2] Compared with appropriate Myrj 45 group.
[3] Calculated by method of Swed and Eisenhart *(9)*.

TABLE 5

Average daily food intake of various groups (for entire 28 weeks of experiment)

Group	Total food	Protein	Average Increase in Body Weight		
			Per day	Per 100 g. food	Per g. protein
	g.	g.	g.	g.	g.
Control	4.6	1.2	0.194	4.2	0.161
5% Myrj 45	4.3	1.1	0.184	4.3	0.167
15% Myrj 45	5.0	1.3	0.204	4.1	0.157
5% Glyceryl Monostearate	4.0	1.0	0.214	5.4	0.214
15% Glyceryl Monostearate	5.0	1.3	0.194	3.9	0.150

apparently due to diarrhea, dehydration, and secondary respiratory infection. Evidence of diarrhea was found only in the 15% Myrj 45 group. This has persisted throughout the experiment.

Results of the histopathological examination of the tissues are summarized in Table 6. It is evident that no consistent pathologic changes were observed in either the liver, kidney, bladder, or testis.

Second Series. *Experimental.* Sixty-seven weanling male hamsters of the Golden Syrian strain were fed a stock pellet ration (rat) for a 6-day "equilibration period." As in the first series, they were housed in individual wire cages, given water *ad libitum*, and carefully weighed. They were kept in a room free of draft at 76–78° F. during the entire study.

After the preliminary 6-day period to allow for adjustment to solid food, animals were reweighed and distributed as equally as possible with respect to body weight into the following groups.

Group I Control—9 hamsters
Group II 15% Myrj 45 with agar—15 hamsters
Group III 15% Myrj 45 without agar—15 hamsters
Group IV 15% Glyceryl-monostearate with agar—14 hamsters
Group V 15% Glyceryl-monostearate without agar—14 hamsters

Exact compositions and caloric values of the diets fed to the 5 groups are given in Table 7. The animals were continued on the respective diets for 22 weeks. Body weights were taken weekly and total food consumption was recorded as before. Animals

TABLE 6

Summary of pathological findings*

Group	Number of animals studied	Kidney	Liver	Bladder	Testis	Comments
Control	3	0	0[1]	0	0	No gross pathology
5% Myrj 45	5	0	+[2]	0	0[3]	No gross pathology
15% Myrj 45	11	0	+[4]	0	0	No gross pathology except diarrhea
5% GMS[5]	4	0	+[2]	0	0	No gross pathology
15% GMS[5]	4	0	+[2]	0	0	No gross pathology

* Legend:
0 = negative.
+ = mild change.
[1] + in one animal.
[2] + in all animals (mild "focal necrosis").
[3] Oligo-spermia in two animals.
[4] + in six animals.
[5] GMS = Glyceryl monostearate.

TABLE 7
Composition of diets
(Second Series)

Constituent	Group				
	I Control	II 15% Myrj 45 with agar	III 15% Myrj 45 without agar	IV 15% Glyceryl Mono-stearate with agar	V 15% Glyceryl Mono-stearate without agar
Casein	24	24	24	24	24
Sucrose	41	32.9	37.9	37.2	41.2
"Vitaminized sucrose"[1]	10	10	10	10	10
Corn oil	15	8.1	6.1	3.7	2.7
Cod liver oil	1	1	1	1	1
Salt mixture[1]	4	4	4	4	4
Dried brewer's yeast	2	2	2	2	2
Agar	3	3	----	3	----
Myrj 45	----	15	15	----	----
Glyceryl Monostearate	----	----	----	15	15
Cal. per gram	4.49	3.91	3.93	4.08	4.15

[1] See Table 1 for composition.

were observed carefully each week for any gross abnormalities or symptoms. Any animals succumbing during the experiment were autopsied and sections of tissues were taken for pathological examination. At the end of the 22-week observation period, the remaining animals were sacrificed, autopsied, and sections of liver, kidney, bladder, and testis were taken for pathological study.

RESULTS

Average body weight values of the 5 groups are given in Table 8. It is evident that the body weights of the 4 groups given the 15% level of emulsifiers increased less rapidly than those of the untreated controls, as would be expected. Growth of the groups of animals given either Myrj 45 or glyceryl-monostearate did not differ much, although average weights of the latter showed a slightly higher trend than those given Myrj 45. Overlapping of values within groups, however, makes these differences of questionable significance. It is interesting that both groups given agar with the emulsifier showed better weight gains than those not given agar. Undoubtedly this is related to a beneficial effect of some "bulk" in the diet of the hamster. The decreases in the average body weights of all 4 emulsifier-treated groups in the later portion of the experiment resulted largely from sharp decreases in body weight in several members of the groups following respiratory infections, a concomitant decrease in food intake, and ultimate death.

Statistical analysis of the data on group average body weights (Table 9) and on the gains in weight (Table 10) of the various groups confirms the above statements. With one exception, the P values exceed 0.05 thus showing no significant difference between either the group average weights or the gains in weight of the various groups. This is confirmatory of the findings in the first series, as shown in Tables 3 and 4.

The one exception, indicating a possibly significant difference in weight, is the P value of 0.044 obtained by comparing the weight gains of the Myrj 45 and glyceryl monostearate groups. This is undoubtedly due to one animal in the Myrj 45 group who developed extreme diarrhea and a resulting low gain in weight (28 g.). However, when the data of the two groups are compared by a different statistical method, that of Swed and Eisenhart *(9)*, no significant difference in weight gain of these two groups is found.

TABLE 8

Average body weight in grams

Group	Initial number of animals	Week												Average increase in body weight	
		0	2	4	6	8	10	12	14	16	18	20	22	Total	Per day
Control	9	45	59	80	98	106	111	112	111	111	111	111	109	64	0.417
15% Myrj 45 + agar	15	45	53	66	76	96	108	106	102	101	96	96	96	51	0.332
15% Myrj 45	15	45	55	69	83	88	100	92	85	88	84	85	92	47	0.304
15% GMS + agar	14	45	54	81	98	111	114	113	107	106	98	98	96	51	0.332
15% GMS	14	45	57	75	91	106	112	111	108	102	96	93	93	48	0.310

TABLE 9

Statistical analysis of group average weights of various groups

		Control	15% Myrj 45	15% Myrj 45 + agar	15% Glyceryl Mono-stearate	15% Glyceryl Mono-stearate + agar
10 weeks	Number of animals	9	11	11	9	11
	Average weight (g.)	111	100	108	112	114
	Standard deviation	±15.2	±12.1	±20.6	±13.1	±8.4
	"t" Value[1]	.....	1.72	0.342	0.145	0.53
					0.635[2]	0.257[2]
	P[1]	.....	0.10	>0.5	>0.5	>0.5
					>0.5[2]	>0.5[2]
22 weeks	Number of animals	8	4	10	6	11
	Average weight (g.)	109	92	96	93	96
	Standard deviation	±17.8	±7.6	±15.7	±10.4	±16.3
	"t" Value[1]	.....	1.67	1.55	1.82	1.57
					0.047[2]	0.000[2]
	P[1]	.....	0.13	0.17	0.08	0.14
					>0.5[2]	>0.5[2]

[1] Compared with control group. For calculation, see Snedecor, *(10)*, pp. 65 and 81.
[2] Compared with appropriate Myrj 45 group.

It is interesting that average body weights of *all* groups of animals used in the present study are lower than those of the first series. Initial average group weights (45 g.) are also less than those of the earlier series (86 g.). However, the average weight gain of the groups of this series was greater than that of the first. No explanation for this difference is apparent, unless it is that the second series was started on experiment at a somewhat earlier age and were therefore carried through the period of most rapid weight gain. The longer initial "equilibration" period used in the first series may also be a factor. There were no significant differences in the basal diets used for the control groups of the two series.

TABLE 10

Statistical analysis of gains in weight of various groups over the ten-week period of growth

	Control	15% Myrj 45	15% Myrj 45 + agar	15% Glyceryl Mono-stearate	15% Glyceryl Mono-stearate + agar
Number of animals	9	11	11	9	11
Average weight gain (g.)	66	55	63	67	69
Actual range	48–82	28–63	28–99	56–93	62–78
Standard deviation	±11.3	±14.1	±21.3	±13.0	±5.3
"t" Value[1]	.....	1.89	0.127	0.69	0.784
				2.45[2]	0.605[2]
P[1]	.....	0.078	>0.5	>0.5	0.46
				0.044[2]	>0.5
P[3]	.....	0.399	0.429	0.762	0.354
				0.135[2]	0.063[2]

[1] Compared with control group. For calculation, see Snedecor, *(10)*, pp. 65 and 81.
[2] Compared with appropriate Myrj 45 group.
[3] Calculated by method of Swed and Eisenhart *(9)*.

TABLE 11

Average daily food intake and weight gain (during ten-week period)

Group	Total food g.	Protein g.	Average increase in body weight			
			Per day	Per 100 cal.	Per 100 g. food	Per g. protein
Control	5.07	1.22	.943	4.05	18.6	.773
15% Myrj 45 + agar	4.33	1.04	.900	4.96	20.8	.865
15% Myrj 45	4.29	1.04	.790	4.53	18.3	.760
15% GMS + agar	5.02	1.21	.985	4.68	19.7	.814
15% GMS	5.30	1.28	.957	4.24	18.1	.747

Table 11 shows average daily food intake and average weight gain per gram food intake of the various groups during the first 10-week period of active growth. Values were obtained by subtracting total food left or spilled during the period from the total amount of diet prepared. Determination of the amount spilled, although done as carefully as possible, entailed some inaccuracies (contamination with urine, etc.). It is evident that the average daily total food intakes of the control and the glyceryl monostearate groups were greater than that of the Myrj 45 groups. Average food intakes of the groups given agar were larger than those not given agar. This parallels the growth rate of the groups, as might be expected. Daily protein intake, of course, parallels the total food intake values. It is interesting, however, that when the weight gain either per gram protein or per 100 g. food intake or per 100 calories is calculated, values for the Myrj 45 groups are higher than those of the glyceryl-monostearate groups. This suggests a better utilization of food for growth purposes by the Myrj 45-fed groups as compared with the glyceryl-monostearate groups. This is rather surprising in view of the fact that more severe diarrhea occurred in the Myrj 45 groups. The data also indicate a better utilization of food protein and calories by agar-fed groups than by non-agar groups.

Numbers of surviving animals at progressive intervals of the experiment are given in Table 12. Only one death occurred in the control group during the 22-weeks of observation. This resulted from blockage of the urinary bladder with a large stone ("phosphate") weighing 83 mg. Little difference in mortality was found in the emulsifier-treated groups. The two groups given the emulsifiers with agar showed similar survival trends and the two groups not given agar were likewise similar. However, it is evident that mortality was significantly less in the two groups given agar. At irregular intervals during the experiment, deaths occurred in these groups due apparently to diarrhea, dehydration, and subsequent terminal respiratory infection. One exception in the GMS + agar group was a death due to urinary bladder blockage from a large phosphate calculus (weight 54 mg.), similar to that found in the control hamster. It might be added that a third stone was found also at the autopsy of another control animal maintained for several weeks on experiment for another purpose after the termination of the emulsifier study.

Table 13 summarizes the pathological findings on sections of liver, kidney, bladder, and testis. *All* sections submitted to the pathologist were by coded number. It will be noted that no significant pathological changes were found in the tissues of animals of any group with the possible exception of the 15% Mrrj 45 + agar group in which sertoli cells were observed in sections of the testes of one animal. This, however, is of questionable significance.

Pathological examination of the tissues confirms the gross observations of the animals at autopsy. The only gross abnormality observed in any of the animals was a possible smaller size of the testes of the 15% Myrj 45 groups. This may be due at least in part to the smaller average gross size of the animal.

With 3 exceptions, to be mentioned later, deaths of animals during the experiment were due to respiratory infection, probably resulting in most cases from diarrhea and subsequent dehydration and lowered resistance to infection. This was particularly true

TABLE 12

Number of surviving animals

Group	Weeks on experiment											
	0	2	4	6	8	10	12	14	16	18	20	22
Control	9	9	9	9	9	9	9	9	9	9	9	8
15% Myrj 45 + agar	15	13	13	13	12	12	12	12	11	11	10	10
15% Myrj 45	15	13	13	11	11	11	11	8	7	7	6	4
15% GMS + agar	14	13	12	11	10	10	10	10	10	10	10	10
15% GMS	14	12	12	11	9	9	8	8	7	6	6	6

TABLE 13
Summary of pathological findings

Group	Number of animals studied	Kidney	Liver	Bladder	Testis	Comments
Control	4	0	0	0	0	No gross pathology[1]
15% Myrj 45+agar	6	0	0	0	0[2]	No gross pathology except diarrhea and possibly slightly smaller size of testis
15% Myrj 45	6	0	0	0	0	As above
15% GMS + agar	6	0	0	0	0	No gross pathology[1]
15% GMS	7	0	0	0	0	No gross pathology

Legend:
0 = negative.
+ = mild change.
[1] Except for large stone found in bladder of one succumbing.
[2] Sertoli cells found in one section.

in the 15% Myrj 45 groups. Pathological examination of lung tissue was taken at autopsy and substantiated this interpretation.

Deaths of one control naimal and one 15% GMS + agar animal during the experiment, and one additional control animal several weeks later were found to be the result of complete occlusion of the urinary bladder by a single large phosphate stone. The stones weighed 83, 54, and 270 mg. respectively. No other stones were found in the bladders or renal pelvis of any other animals. This point was carefully checked at autopsy in *all* cases, both the kidneys and the bladder of *each* anmial being opened and examined thoroughly in strong light using a magnifying glass.

No evidence of tumors or other abnormal growths was found in any animal either during the course of the experiment or at autopsy. Special search for any such abnormalities was made in all animals.

DISCUSSION

Results of the foregoing two series of studies thus give no indication of any distinct toxic effect, other than diarrhea, of either polyoxyethylene-8-stearate or glyceryl-monostearate when fed at either 5 or 15% levels to male hamsters, for a 22 to 28-week period. The growth rate at the 15% level was somewhat less than that of control groups. The differences were not statistically significant. Moderate to severe diarrhea was noted, particularly in the polyoxyethylene-stearate group. This was lessened by the inclusion of agar in the diet. No consistent gross or microscopic pathology was found in the liver, kidney, bladder, or testis of any of the animals although the testes of the 15% polyoxyethlene-stearate-fed group appeared somewhat smaller than those of the other groups of animals. This may be related to the lower body weights of this group of animals. Deaths during the experiment in the emulsifier-fed groups appeared to be due to respiratory infection perhaps resulting from lowered resistance related to severe diarrhea and subsequent dehydration.

Since no toxic symptoms, including diarrhea, were observed in the groups fed the 5% level of the emulsifiers, it is evident that the "no effect" level must be between 5 and 15%. Diarrhea was frequently observed at the 15% level. No attempt was made to determine the maximum level tolerated without diarrhea.

In general, the present findings agree with those of other investigators in rats and cats *(3, 4, 5, 7)* and hamsters *(1, 6)* but appear somewhat at variance with those of several others *(2, 8, 11)*. The apparent differences in results, at least with hamsters, may be related to either strain differences in animals used or, more likely, to the effects of unsuitable basal diets due to the present incompletely understood nutritional requirements of this species.

SUMMARY

Data obtained from studies on the two series of animals may be summarized as follows:

Administration of 5 or 15% levels of polyoxyethylene-8-stearate (Myrj 45) and of glyceryl monostearate to weanling male hamsters for periods of 22 to 28 weeks resulted in only slight effects on growth and ultimate body weight as compared with those of unsupplemented controls. Average body weights of the various groups at the 10- and 22-week periods of both series and the gains in weight for the 10-week growth period were not significantly different when evaluated by two differing methods of statistical analysis. Addition of agar to the diet had some beneficial effect in lessening the moderate to severe diarrhea observed, particularly in the animals given the 15% level of polyoxyethylene-8-stearate. Growth was increased slightly also.

No striking differences were found in the food intake of the various groups of either series. A somewhat lower average daily food intake was found in the group (second series) fed 15% polyoxyethylene-8-stearate. However, the weight gain per 100 gram of food or per gram of protein or per 100 calories consumed was somewhat higher in the Myrj 45-fed groups of hamsters than in the other groups. Agar improved somewhat the utilization of food by the emulsifier-treated groups.

Little difference in mortality was found between the animals fed the two emulsifiers. Those groups given agar, however, survived somewhat longer than those not given agar.

No consistent gross or microscopic pathology was found in the tissues (liver, kidney, bladder, and testis) of any of the animals of the various groups, except for the possible smaller gross size of the testes of the group fed 15% polyoxyethylene-8-stearate. Calculi of the urinary bladder were found in only 3 animals (two controls and one 15% glyceryl-monostearate plus agar).

Deaths during the experiment in the emulsifier-fed hamsters were due to respiratory infection apparently related to diarrhea and subsequent dehydration and lowered resistance.

From the foregoing results, the general conclusion appears warranted that no distinct toxic effects resulted from the feeding of 5 or 15% levels of either polyoxyethylene-8-stearate or glyceryl-monostearate, other than diarrhea and a resultant dehydration and occasional weight loss in the 15% level groups, to young male hamsters for periods of 22 to 28 weeks.

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NONENZYMATIC DISCOLORATION OF GREEN BEAN PURÉE. II. STUDIES ON THE THERMAL DISCOLORATION OF ACETONE-EXTRACTED TISSUE [a, b]

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In a previous study *(14)* comminuted green beans, which had been thoroughly extracted with acetone to remove the pigments present, became darker when heat processed. This was attributed, in part, to the occurrence of a polyphenol type of reaction. This study has been extended in an attempt to shed new light upon the factors causing the discoloration.

EXPERIMENTAL

GREEN BEAN PURÉE

Preparation. Fresh green beans (*Phaseolus vulgaris*, unidentified variety) locally purchased, were washed, trimmed, and cut. Comminuting was accomplished using a Fitzpatrick Mill with a No. 2 fine mesh screen.

Darkening rate. Tubes of prepared green bean purée were processed in a small steam heated retort, similar to that described by Schmidt *(12)*, equipped with a Brown Electronik Strip Chart, Six-point Potentiometer, according to the schedule in Table 1. After processing, puree was extracted with acetone to reveal color of the tissue itself without interference of the chlorophylls and their degradation products.

Color measurement. Color of tissue was evaluated by reflection measurements using either the Photovolt Reflection Meter, Model 610, or the Hunter Color and Color-difference Meter. With the former, a porcelain capsule, 34 mm. in diameter and 4 mm. deep, covered with a piece of clear colorless glass, was used as a sample holder. Measurement was taken by placing the search unit upon the center of the sample. The instrument was used with a tri-stimulus green filter and was standardized with Munsell N/9 and N/4 paper standards in the experiments carried out to evaluate the effect of the solids content. The instrument was set at 100% with a white enamel standard (Y = 88.5) when the effects of process time and temperature were studied. The Hunter Color and Color-difference Meter was employed for all other color measurements. With this instrument the tissue was placed upon a glass plate. Standardization was carried out with a white enamel standard (Rd 83.0, a −2.0, b 2.4) using the small area illumination. Consistency of the sample was critical in the color determination. Therefore, for samples having a high solids content, enough water was added to yield a slurry which would just mound up without separation of the liquid. At the other extreme, with very low solids samples, sufficient liquid was removed to give the same consistency.

DECOLORIZED GREEN BEAN TISSUE

Preparation. Valentine variety green beans, blanched for 4 minutes in boiling water, were comminuted by the previously described method. Blanching was necessary as the tissue prepared by the method previously reported *(14)* was found to give a strong test for peroxidase using o-phenylenediamine as described by Reddi, Esselen and Fellers *(10)*.

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Purée was extracted batch-wise with acetone, until solvent no longer removed any color. Following filtration by suction, to remove most of the acetone, the white tissue was dried in vacuum and stored in sealed containers at about −15° C. until used.

Sealed tube studies. The method of Velice *(13)* was used to prepare sealed tubes for studying the darkening of the reconstituted purée. Lipless, 18 x 150 mm., pyrex culture tubes were filled to within 30 mm. from the top, and were drawn out to fine capillaries. Headspace gas was adjusted to 100% nitrogen. Processing was effected by placing the tubes either in an oil bath for 30 minutes at 121° C. or in an autoclave for 6 minutes at 121° C. Tubes were cooled in cold running water.

Tissue fractions. The cellulose of solvent extracted tissue was isolated by Bennett's modification *(3)* of the Norman-Jenkins method *(8)*. Holocellulose was obtained by the procedure of Whistler, Bachrach and Bowman *(15)*, except that 2.5 g. of sodium chlorite, 0.3 ml. of glacial acetic acid, and 200 ml. of distilled water were used on 5 g. of the tissue. This proportion was found to give a pH value of 4.5 which is optimum for the reaction. Lignin was obtained by sulfuric acid hydrolysis of the beans by the method of Norman and Jenkins *(8)*.

Solids content adjustment. Solids content was adjusted to desired level by adding calculated weights of distilled water to acetone-insoluble solids of green beans. Above 20% solids, the liquid was added to the dry tissue and triturated with a glass mortar and pestle until the mixture was homogeneous. Below this concentration of tissue, adequate mixing was attained by shaking the dry material with added water. Prepared material was weighed into tubes. Three replicates were prepared for each solids level, except that 6 replicates were used at the 20% level. The sealed tubes were processed for 30 minutes at 121° C.

Adjustment of pH. The pH was adjusted to the desired level by the addition of either 0.5M trisodium phosphate and/or 0.5M phosphoric acid. The pH was determined by a Beckman Model G pH meter. When the desired pH value was obtained, buffer previously adjusted to this pH was incorporated to give a solids content of 3.5%. Three replicates were used for each pH level, and the tubes were heated for 30 minutes at 121° C.

REDDENING

Effect of acid. Acetone-extracted green bean solids, prepared as described in the preceding section, were adjusted to pH 2.5 with each of the following acids: hydrochloric, sulfuric, phosphoric, malic, citric, acetic and lactic acids. A solids content of 3.5% was used. Prepared material was filled into tubes which were processed for 30 minutes at 121° C.

Effect of time and temperature. Several sets of tubes were processed at 3 different temperatures for various lengths of time. Decolorized green bean tissue was adjusted to pH 1.5 using a minimum of M H_3PO_4 and 0.5M Na_3PO_4. When this pH was attained, enough of the prepared buffer at pH 1.5 was added to give 3.5% solids. The tubes sealed with nitrogen as the headspace gas, were processed in a dibutyl phthalate bath at the temperature required for the desired length of time. After processing, tubes were cooled in a stream of cold water.

ANTHOCYANIDINS AND LEUCOANTHOCYANIDINS

Identification of anthocyanidins. Red pigments which were formed were partially extracted by 2M and 0.5M phosphoric acid and 2M phosphoric acid-acetone (1:1). After concentrating the red solution in a rotating vacuum evaporator below 45° C. until free of acetone, red pigments were extracted from the aqueous phase with n-amyl alcohol. The method of Robinson and Robinson *(11)* was used for preliminary purification. Subsequent purification and concentration was effected employing the technic of Markakis *(7)*. Ascending chromatography with n-butanol-2N HCl, as recommended by Bate-Smith *(1)*, was used.

Extraction of leucoanthocyanidins. The following solvents were evaluated as potential extractants for leucoanthocyanidins of acetone-insoluble green bean tissue: water, 2% formic acid, 10% formic acid, methanol, methanol containing 1% HCl and 10% HCl. The test for extracted leucoanthocyanidins involved heating the filtered solvent adjusted

to give a concentration of 2N HCl, for 20 minutes in a boiling water bath. A positive test was indicated by the production of a pink to red solution. Intensity of color was taken as being proportional to concentration of the pigments.

Processing studies on leucoanthocyanidins. Three sets of tubes were prepared. The first, used as controls, contained holocellulose plus buffer or distilled water. A second set contained holocellulose and pure cacao leucocyanidin[4] which was dissolved in a known volume of water: a quantity equal to 4.0 mg. leucocyanidin was added to each tube containing holocellulose (0.5 g.). The last group had holocellulose and a crude fraction of leucoanthocyanidins obtained by extraction of acetone-insoluble bean solids with 2% formic acid for about 2 hours. Each set was subdivided into 3 different pH levels: pH values of 2 and 8 were obtained by the use of buffers (as described in the previous section) and a third pH level was obtained by the use of distilled water. Holocellulose was used at a 3.5% solids level in all cases.

Also two sets of tubes were prepared in which holocellulose was not used. These tubes contained the crude fraction of leucoanthocyanidins or pure leucocyanidin made up in distilled water or buffer of pH 2 or 8.

RESULTS AND DISCUSSION

GREEN BEAN PURÉE

Darkening rate. Green bean tissue undergoes a discoloration during heating. The extent of this discoloration is proportional to the amount of heat received during processing. To express the heating times and temperatures (Table 1) in one unit for the purpose of establishing any possible correlation between this and the reflectance loss, it was necessary to convert the time-temperature relationship to another term. F_o units were used as a common denominator. An F_o unit, as ordinarily used for calculating thermal process times for canned foods, means "sterilizing value", or the amount of lethal heat in terms of minutes at 121° C. For the purpose of these calculations, F_o was used as the "destructive effect". An arbitrary darkening rate curve was assigned a slope of 10° C., that is, it would take 10° C. (on the linear abscissa) to traverse a log-cycle of time (plotted on the ordinate) when the time to produce a given amount of darkening at 121° C. is one minute on this curve. Using the general method *(4)* of evaluating processing requirements, the destructive effect of each process was calculated. Data shown in the table are arranged in order of ascending F_o units. Reflectance measurement is of limited accuracy for measuring darkening; however, this procedure was used because it was the only method available for objective evaluation of the insoluble color. The correlation coefficient between F_o and reflectance values was calculated and found to be 0.83, which is significant at the 1% level. Thus, there was a direct relationship between the severity of darkening and the amount of heat received.

Examination of these data reveals that there were occasional extreme variations where an increase in F_o did not result in the anticipated decrease in reflectance. Perhaps these irregularities were due to the comparatively long come-up times required to heat the contents of the tube to retort temperature. By the time the tissue had reached the desired temperature, a major part of the loss in reflectance had already occurred. Therefore, further heating showed only a relatively small change. By the use of a very rapid heating rate this difficulty might not have occurred.

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[4] Isolated from cacao bean, and kindly supplied by Dr. W. G. C. Forsyth, Colonial Microbiological Research Institute, Trinidad.

TABLE 1

Effect of process time and temperature on the reflectance of decolorized green bean tissue [1]

Process		Reflectance[3]	F_o[4]
Time[2]	Temperature		
(min.)	*(° C.)*	(%)	
Control		43.0	0
0	116	43.5	0.10
0	121	52.5	0.35
3	116	39.0	1.05
6	116	46.0	1.42
10	116	42.5	2.45
3	121	47.0	2.88
15	116	27.5	3.88
6	121	42.5	6.71
0	132	39.5	9.00
10	121	40.0	12.3
15	121	36.0	16.1
2	132	30.5	32.5
5	132	21.5	76.2
8	132	18.5	86.0
0	144	35.0	130
12	132	18.5	156
15	132	18.5	201
1	144	27.5	342
3	144	22.5	673
5	144	21.0	990

[1] After processing, the green bean purée was extracted with acetone before the reflectance measurements were taken.

[2] Holding time at retort temperature; at 0 minutes the tubes were brought up to the retort temperature indicated and immediately cooled: at 116° C. come-up-time to retort temperature was about 1.5 minutes, at 121° C. it was 1 minute, at 132° C. it was 2 minutes, and at 144° C. it was about 3 minutes.

[3] Reflectance measured with a Photovolt Reflectometer, Model 610, equipped with a tri-stimulus green filter, instrument set at 100% reflectance with a white enamel standard (Y = 88.5).

[4] F_o calculated from heating data (recorded on a thermograph) using a "z" value of 18 to determine the destructive effect.

DECOLORIZED GREEN BEAN TISSUE

Tissue fractions. Successive extractions of the acetone-insoluble matter of green beans with boiling water, 0.5% ammonium citrate solution, 95% ethanol, ethanol-benzene (32:68), isobutanol and ethyl acetate did not materially decrease the capacity for discoloration when heated. This is additional evidence for the theory postulated in the previous paper; i.e., that the darkening involves the tissue itself, and not the soluble components. Consequently, the principal insoluble fractions—namely, cellulose and holocellulose—were isolated and tested. Neither one became appreciably darker when processed for 6 minutes at 121° C. Data are shown in Table 2.

Since lignin was the major constituent removed from extractive-free tissue in the isolation of holocellulose, its role in the darkening was further studied. When green bean lignin was added back to holocellulose and the mixture was processed, no darkening occurred. This apparent lack of reactivity of lignin with holocellulose might be due to the nature of this lignin. First, it is thought that the severe conditions which were employed in the isolation of lignin caused considerable changes in its reactive groups, resulting in a relatively

TABLE 2

Effect of chemical treatments upon the thermal darkening of acetone-extracted green bean tissue

Fraction	Extraction device	Time (hrs.)	Reflectance[1]		
			Un-processed	Processed[2] headspace gas:	
				Nitrogen	Air
Original (Control)	.....	....	41.5	25.0	23.0
Extracted with ethanol	Soxhlet	4	50.5	23.5	40.0
Extracted with water	Soxhlet	4	17.0	.....	.....
Extracted with water	Percolator	....	33.5	.....	.....
Extracted with water and organic solvents	Percolator	....	37.5	24.5	23.5
Holocellulose	.....	....	46.0	43.5	42.0
Cellulose	.....	....	38.0	.....	36.5

[1] Reflectance measured with a Photovolt Reflectometer, Model 610, equipped with a tri-stimulus green filter, instrument set at 100% reflectance with a white enamel standard (Y = 88.5).
[2] Processed at 121° C. for 6 minutes.

inert lignin. Second, the insolubility of the lignin and the holocellulose under the experimental conditions which were used and the resultant failure to establish intimate contact between them might have prevented any interaction.

Effect of solids. Solids content affects the intensity of the discoloration. The latter is at a minimum at high and low solids content and apparently reaches a maximum over the range of 50 to 80% solids. This is illustrated in Figure 1. At the lower solids levels, discoloration was gray-purple, and at the higher solids contents, discoloration was gray-brown. At solids contents above 50%, it was difficult to obtain a uniform distribution of the moisture due to the extremely hygroscopic nature of the bean tissue. This may have caused the erratic results obtained at the higher levels. However, based upon the published reports of other investigators of browning reactions *(6, 9)*, it would not be illogical to assume that the browning tendency should reach a peak at a certain concentration, level off with further increases in solids, and then decrease.

Although variations in come-up-time (length of time required for a given tube to reach processing temperature) might have occurred due to differences in solids content, corrections were not made since they would have accounted for only a small percentage of the total heating time.

Effect of pH. The color of unheated acetone-extracted tissue changes as the pH is adjusted. Above pH 4.0, the tissue becomes darker and below, it becomes lighter. Part of the change was due to the effect of pH on the holocellulose. Processing studies with pH as the variable indicate that at, or near, pH 4.0 there is maximum stability of color during heating; below this pH, the tissue becomes progressively redder with increases in heating time and with decreases in pH. Above pH 4, the darkening assumes a brown coloration: the intensity of the brown color is related to heating time and pH, as shown in Figure 2. At pH 8 a significant amount of the dark color can be attributed to the reaction of the holocellulose to heat and alkali. Additional discoloration may have been due to leucoanthocyanidins as evidenced by the

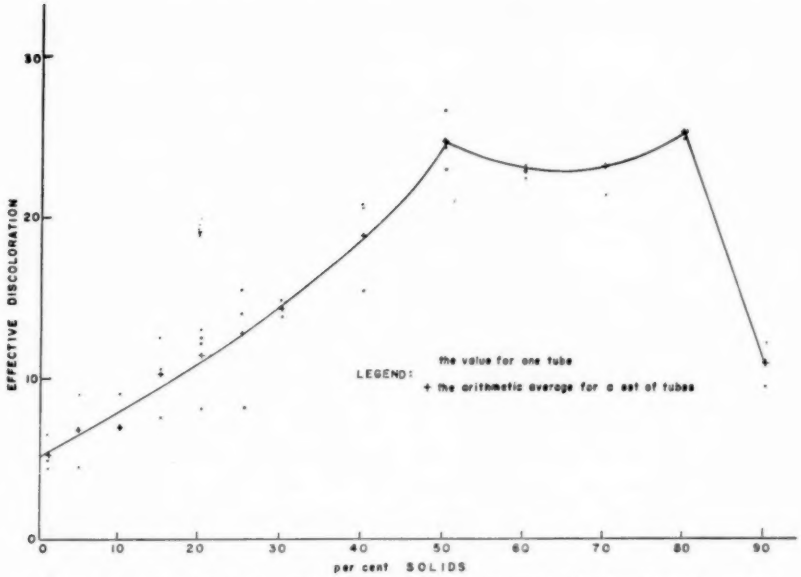

Figure 1. The effect of solids content on the thermal discoloration of acetone extracted green bean tissue processed for 30 min. at 121° C. (Effective discoloration is the per cent reflectance after processing subtracted from the per cent reflectance before processing.)

fact that these, by themselves, discolor under this condition. Because of refinements introduced here, notably the use of a buffer system and a longer heating time, these data are somewhat different from those reported in the previous paper of this series *(14)*.

Tubes were selected at random from the processing studies on the effect of time and temperature, solids and pH. When these tubes were opened, the headspace gas was swept out with nitrogen into $Ba(OH)_2$ to determine if CO_2 was produced. In all cases this qualitative test indicated that CO_2 was present. Also, production of color was accompanied by formation of an unpleasant odor.

REDDENING

Effect of acids. To determine whether or not reddening of the decolorized green bean tissue cooked with phosphate buffer below pH 4 was due to the buffer itself, the tissue was heated with different acids at pH 2.5. Cooking the tissue with hydrochloric, sulfuric, phosphoric, malic, citric, acetic or lactic acids resulted in a redness which was of the same intensity with the different acids. This was interpreted as conclusive evidence that the production of red color was due to the effect of the low pH and heat.

Effect of time and temperature. Minimum time required to produce the first sign of pinkness as indicated by a positive "a" value on the Hunter Color and Color-difference Meter, was 8 minutes at 100° C.; 5 minutes at 110° C., and 4 minutes at 121° C. These heating times are not corrected for heating or cooling times: they represent the actual time the tubes remained in the oil bath.

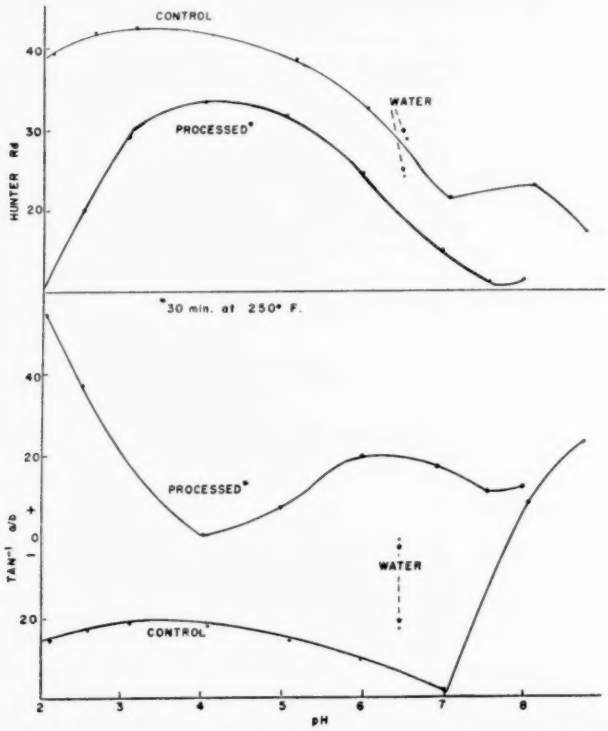

Figure 2. The effect of pH on color of acetone extracted green bean tissue. (Tan^{-1} a/b when highly positive it is red, as it approaches zero it is yellow and when negative it becomes progressively greener.)

Redness could not be produced in holocellulose under the conditions just described, indicating that the colorless precursors were either extracted or destroyed in the isolation of the former.

Experiments designed to determine the ubiquity of the "pinking" reaction showed that the acetone-insoluble peel-less pulp of McIntosh apples, d'Anjou pears and Golden Jubilee peaches turned red when processed at a low pH in sealed tubes for 30 minutes. But Blue Hubbard squash, an unknown variety of peas, and carrots, thought to be Imperator, did not do so. Upon comparing the tissues which were cooked with water, to those cooked with phosphate buffer at pH 2 or less, it was observed that those tissues which had turned red under acid conditions also turned brown when processed with distilled water. The other tissues became somewhat darker but showed no sign of browning.

ANTHOCYANIDINS AND LEUCOANTHOCYANIDINS

Identification of anthocyanidins. Paper chromatography of the red pigments isolated from beans cooked with acid revealed that two bands of red pigment of equal intensity were present in about equal concentration.

The Rf values of 0.65 and 0.30 were indicative of cyanidin and delphinidin: spot tests were used to confirm the identifications. This finding agrees with the Bate-Smith *(2)* identification.

Extraction of leucoanthocyanidins. Methanol was the only liquid tested that was capable of extracting leucoanthocyanidins when the solvent remained in contact with the tissue for 18 hours at room temperature. Acidified methanol produced redness which indicated that the leuco-forms were extracted but were converted to their red forms in the process. Methanol or distilled water were effective when used in a Soxhlet apparatus. Two per cent formic acid solubilized some chromogenic matter only when the tissue was left in contact with this liquid for two hours in a steam bath.

Processing studies with leucoanthocyanidins. Anthocyanin pigments have been reported to cause the black or brown discoloration of canned blackeye peas *(5)*. Green bean seeds were found to brown more extensively than the pods *(14)*. It is thought, in light of the present investigation, that the bean seed discoloration is quite similar to that occurring in peas. Further support for this theory is offered by the fact that leucoanthocyanidins are present in the testa of the young bean seeds and none is present in the pods *(2)*. These observations and the data presented in a previous section are indirect evidence that the presence of the colorless or leuco-form of the anthocyanidins, is conducive to increased darkening.

To obtain more conclusive information, the precursors to the red pigments were added to holocellulose. When adjusted to pH 2.0 and when distilled water was added, the holocellulose tubes containing added chromogens became somewhat darker. This change was more pronounced in the tubes containing pure leucocyanidin. At pH 8 the change was even more distinct: the tube with the pure leucocyanidin became tan-colored, and the one with the crude fraction became slightly yellow. Since the crude fraction was more dilute with respect to leucoanthocyanidins, the holocellulose with this added material did not darken as much as when the purer material, cacao leucocyanidin, was added. The liquid controls, i.e., those without holocellulose, containing pure leucocyanidin also showed similar changes, except that at pH 8 the change was toward pink rather than tan. Upon processing, all tubes became darker when compared to their unprocessed controls, and those tubes with added chromogens were generally darker than the tubes with just holocellulose and liquid. The tube which did not darken more than the processed holocellulose control, was the one processed at pH 8 in the presence of crude leucoanthocyanidins. As with the unprocessed samples, the darkest processed tubes at the three different conditions of pH, were the ones with pure leucocyanidin added. These data are shown in Table 3.

Heated tubes containing liquid without insoluble solids also darkened with the exception of those containing crude leucoanthocyanidins at pH 2, and those with distilled water. These did not change visibly. The evidence seems to indicate that leucocyanidin when heated under the pH conditions used becomes much darker. The crude fraction when added to the tissue, and processed, causes increased thermal darkening.

An additional finding which supports the contention that leucoanthocyanidins are implicated in thermal discoloration is offered by the fact that

TABLE 3

Effect of leucoanthocyanidins upon the thermal discoloration [1] of holocellulose processed for 30 minutes at 121° C.

Holocellulose						Holocellulose plus cacao leucocyanidin						Holocellulose plus crude green bean leucoanthocyanidins					
Treatment	pH		Rd	a	b	Treatment	pH		Rd	a	b	Treatment	pH		Rd	a	b
	Prepared	Final					Prepared	Final					Prepared	Final			
Unproc.	2.0	1.88	38.9	−2.9	2.7	Unproc.	2.0	1.92	35.6	−0.7	6.6	Unproc.	2.0	1.88	36.9	−2.9	3.8
Proc.	2.0	2.02	28.1	−2.6	9.3	Proc.	2.0	2.08	20.4	3.4	11.4	Proc.	2.0	2.48	23.0	−1.6	11.2
Unproc.	5.47	5.13	36.6	−2.7	2.7	Unproc.	5.38	4.57	30.6	−0.8	4.9	Unproc.	2.88	2.67	39.3	−2.7	4.8
Proc.	5.47	4.11	25.1	−2.8	8.5	Proc.	5.38	4.40	19.8	−0.5	10.1	Proc.	2.88	3.06	25.1	−0.8	11.9
Unproc.	8.0	7.87	25.7	−3.3	5.2	Unproc.	8.0	8.15	8.2	3.9	14.1	Unproc.	8.0	7.98	21.9	−3.2	9.0
Proc.	8.0	7.68	11.4	9.9	17.0	Proc.	8.0	7.69	7.2	4.7	13.1	Proc.	8.0	7.87	15.0	9.9	15.2

[1] Color evaluated by the Hunter Color and Color-Difference Meter using small area illumination, sample placed directly on the glass plate, and instrument standardized with a white standard (Rd 83.0, a −2.0, b 2.4).

green beans which had the testas removed from the seeds prior to processing—to insure that no leucoanthocyanidins were present—did not darken as much as the whole green beans.

SUMMARY

Of the factors studied in relation to the thermal discoloration of acetone-extracted green bean purée, the most significant appears to be the role of the colorless precursors of the anthocyanidins present as contributors to the discoloration. The extent of the darkening is dependent upon the temperature of heating and the pH. Other conclusions derived from this study on acetone-extracted green beans include:

1. The extent of process browning of green bean purée is directly related to the time and temperature of heating.

2. Components contributing to process discoloration are not sufficiently removed by any of the following solvents to result in a substantial decrease in darkening: boiling water, 0.5% ammonium citrate, 95% ethanol, ethanol-benzene, isobutanol, ethyl acetate and acetone.

3. Process discoloration is most severe in the range of about 50 to 80% solids. It is at a minimum at lower or higher levels.

4. Process browning becomes more severe above pH 4. Below this pH, the color becomes red with processing. Maximum color stability is around pH 4.0.

5. The color of unprocessed tissue is dependent upon the pH.

6. Acetone-insoluble solids of apples, pears and peaches become red when processed at pH 1 with phosphate buffer. Peas, carrots and squash do not turn red when similarly treated.

7. Acetone-insoluble solids of apples, pears, peaches, and green beans become brown when they are processed with distilled water. This may be due to the presence of the anthocyanidins' precursor(s).

8. Reddening of bean tissue can be produced by any of a number of acids if the pH is sufficiently reduced.

9. Minimum time required to produce the first sign of pinkness of the tissue was 8 minutes at 100° C., 5 minutes at 110° C., and 4 minutes at 121° C.

10. Holocellulose does not become red when heat-processed. Neither does this substance become appreciably darker when autoclaved for 6 minutes at 121° C. It does become slightly darker when heated at 121° C. for 30 minutes.

11. Two red pigments are produced in about the same concentration when green bean tissue is cooked with acid. They are delphinidin and cyanidin.

12. Colorless precursors of anthocyanidins are extractable with hot 2% formic acid or hot methanol.

13. Crude leucoanthocyanidins from green beans and pure cacao leucocyanidin both discolor when processed.

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EFFECTIVENESS OF SORBIC ACID IN PRESERVATION OF DAMP WHEAT

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Drying wheat to a "safe moisture level" is considered the most effective method of preventing deterioration in damp wheat. The safe moisture level of wheat above which microorganisms may cause damage is roughly equivalent to 65–70% relative humidity, but the exact level depends on the kind, soundness and history of grain, conditions of storage (temperature, weather, and expected term) and nature of the microorganisms involved.

Many chemicals have been tried for control of fungal activity in grain storage, but the commercial feasibility of chemical treatment has been considered doubtful. The problem is complicated since dusting fungistatic agents on grain generally inhibits the growth of the external microflora but has relatively little or no influence on fungi in the subepidermal layer *(8)*. In some instances, use of fungistatic agents has been reported to inhibit mold growth and the development of fat acidity in damp wheat but did not prevent deterioration in baking quality and spoilage due to abnormal odors. There is, therefore, a need for a more effective, nontoxic fungistatic agent for damp wheat, one that would not have any adverse effect on the final product. Further characteristics required in such a fungistatic agent are low cost and harmlessness from the point of view of expected final use of the grain as animal or human food.

Results of studies summarized in this report show the extent to which sorbic acid can meet these requirements.

Several workers studied the inhibition of fungi *in vitro* by sorbic acid with regard to the extent and selectivity of inhibition *(3)*. Species of *Aspergillus* and *Penicillium*, isolated from tomato and strawberry fruits were shown *(2)* to exhibit great tolerance towards sorbic acid, when grown on tomato juice. On testing the action of sorbic acid on pharmaceutical preparations, growth of *Aspergillus miger* and an unidentified *Penicillium* species were inhibited by concentrations of 0.05%.

Sorbic acid is widely used as a fungistatic agent in a variety of foodstuffs. It is regarded *(7)* as harmless, non-toxic and relatively economic in use. Moreover, it does not impart, at the levels needed for fungal inhibition, any taste or odor to the finished product.

MATERIALS AND METHODS

A sound sample of U. S. hard red winter wheat was used in this investigation. The cleaned wheat had a test weight of 68 lb./bushel (Imperial); moisture content of 12.5% and protein content of 12.4%.

A. Inhibition of fungi infecting wheat by sorbic acid.

Fungal cultures. The following mold cultures were tested: *Aspergillus niger, A. flavus, A. ochraceus, A. candidus, A. amstelodami, Penicillium chrysogenum* and *Alternaria tenuis.*

The mold cultures were inoculated aseptically into a malt-salt broth, incubated for 3 days at 22° C., diluted with 0.5% sterile salt solution and added to sterile wheat.

Heat sterilized wheat. Wheat was moistened in 10 g. lots to 17% moisture in small Erlenmeyer flasks, plugged with cotton and sterilized for 15 minutes at 15 lbs. The next day the samples were moistened aseptically by a dilute solution of sodium sorbate to various levels of moisture and sorbic acid content.

Sorbic acid used in this study was kindly supplied by the Union Carbide International Company.

In one series of experiments the inoculation of mold cultures was made 4 hours after addition to sodium sorbate; in the second series inoculation was made one week after treatment with sorbate. The flasks were incubated for 7 days at 30–31° C., shaken daily, and at the end of the incubation period visual appearance of fungal growth was recorded. Extent of growth is indicated by "–" for no growth and up to "++++" for heavy growth.

B. Inhibition of fungal growth on damp wheat.

Part of the wheat was damped to moisture contents of 15, 18, 20 and 25%, respectively. Samples, together with some of the 12.5% moisture wheat, were placed in 2½ liter narrow-mouthed glass bottles holding 1½ kg. lots. Addition of water to the 25% moisture level was made in two stages at an interval of 4 hours. For addition of sorbic acid in the solid state, the damp wheat has been cold conditioned for 8 hours, removed from the bottles, dusted with sorbic acid, returned to the containers and thoroughly mixed. Sorbic acid in solution was added in the form of a 10% sodium sorbate stock solution, diluted so as to constitute the whole liquid added for damping the wheat.

In choosing the level of sorbic acid to be applied, the investigator was guided by results of preliminary experiments on the effect of sorbic acid (powder form) added to commercially milled, untreated long patent flour—a composite of hard red winter and soft red winter wheat. Results of this experiment are summarized in Table 1. The experiment showed that high levels of sorbic acid in flour—above 0.05%—are injurious to bread quality and loaf volume.

TABLE 1

Effect of sorbic acid, added to commercially milled flour, on loaf volume

Level of sorbic acid	Loaf volume
as % of flour	*cc*
None	1875
0.050	1850
0.075	1650
0.1	1375
0.2	1300
0.4	1175

The mouths of the bottles were closed with cotton wool plugs. The bottles were shaken daily to help maintain uniform moisture distribution and at the same time the samples were inspected for any visual change in appearance due to mold growth. The samples were kept for 5 weeks at room temperature (10–20° C.) and subsequently for one week in a cabinet in which a temperature of 30–32° C. and relative humidity of 95–100% were maintained. At the end of the whole storage period the following examinations were applied:

Milling characteristics. Before milling the lots were spread on paper sheets and air dried over a period of 72 hours, drying being facilitated by use of a fan. The wheat dried to about 14% moisture, was damped to 15.5% moisture, tempered overnight, and milled on a Bühler laboratory mill. Throughout the course of the investigation the conditions of milling, i.e. setting of rolls, rate of feed, etc., were kept uniform in order to reveal any differences in milling characteristics due to mold growth. Though effort was made to maintain uniform milling, some minor variations in grinding are inevitable. Although

they are believed to be small, they must be allowed for when interpreting flour and bread quality data. Yields of flour are reported as percentage of the milled—total—products.

Moisture content. A 10 g. sample was heated for 1½ hour in the case of wheat and 1 hour in the case of flour in a semiautomatic Brabender oven at 130° C.

Fat. Fat was extracted with petroleum ether (b.p. 40–50° C.) in a Soxhlet apparatus.

Fat acidity. Fat acidity was determined by the method of Zeleny and Coleman *(11)*.

Mold counts. Counts were made by the procedure outlined by Christensen *(4)*.

(Moisture, fat, fat acidity and mold count determinations were made on wheat ground through a 30 mesh sieve, in a Micropulverizer).

Ash. Ash was determined on a 5 g. sample incinerated overnight at 580° C. in a silica crucible.

Diastatic activity. Diastatic acivity was determined on a 5 g. sample as outlined in *Cereal Laboratory Methods (1)*.

Protein. Protein was determined on a 1 g. sample by the method outlined in Cereal Laboratory Methods *(1)*.

Flour color. Flour color was measured with the Kent-Jones and Martin Colour Grader *(5)*.

Test weight. The *test weight* determination was made with an Avery apparatus.

Farinograms. Farinograms were obtained by mixing 50 g. of flour in a small bowl (64 r.p.m. drive) for 15 minutes with distilled water to give maximum dough consistency centered about 500 Brabender unit line. Farinograph absorption is reported as the amount of water which was added to a flour (14% moisture basis) to give the mentioned consistency. Dough development time was read off from the farinograph curves. Consistency drop figures were determined on a dough mixed at the predetermined mobility until attainment of maximum consistency. The dough was subsequently rested in the covered Farinograph bowl maintained at 30° C. for 3 hours. At the end of this time the dough was mixed for 3 minutes and change in consistency recorded.

Extensograph curves. To obtain extensograph curves doughs were made from 300 g. flour, 6 g. salt and the amount of distilled water found from the farinograph absorption test, less 3.0 percentage units to compensate for the added salt. This series of experiments was made on a commercially milled, untreated bakers' long patent flour. Doughs were mixed for 1 minute in a large farinograph bowl, rested for 5 minutes and remixed until the curve was centered about the 500 unit line. Curves were drawn for duplicate 150 g. doughs after 45, 90, and 135 minutes' rest periods.

Baking tests. The straight dough method was employed in the baking tests and doughs were of such size as to yield commercial loaves of 500 g. The formula for the dough included flour, water, 3% compressed yeast and 1% salt (both percentages on flour basis); the dough was mixed in a Junior Hobart Mixer for 5 minutes at medium speed. Moulding was done by hand and optimum absorption was determined by subtracting 4 percentage units from the farinograph absorption (minor adjustments were made if the handling properties of the dough required it). Fermentation was kept uniform at 110 minutes. Proofing time was chosen so as to secure optimum conditions as judged by the operator and varied between 35 and 75 minutes. The dough was fermented in a cabinet maintained at 30–31° C. and 95–100% relative humidity. The tinned bread was baked for 50 minutes at 210° C. Loaf volume was measured the next day and remarks added where necessary.

RESULTS AND DISCUSSION

A. Inhibition of fungi infecting wheat by sorbic acid.

Inhibition of fungal growth on mold-inoculated, sterile wheat, treated with sorbic acid, is summarized in Table 2.

The findings show that for complete inhibition of fungal growth on mold inoculated wheat, an addition of sorbic acid at a level of 0.2% is needed.

TABLE 2

Inhibition of fungal growth on mold inoculated sterile wheat by treatment with sorbic acid

Species	Sorbic acid, %				
	0	0.05	0.075	0.10	0.20
Moisture content 19% inoculated 4 hours after addition of sorbic					
A. niger	++	−	−		
A. flavus	++++	++	−		
A. ochraceus	++	−	−		
A. candidus	++++	+	−		
A. amstelodami	+++	+	−		
P. chrysogenum	+	−	−		
Alt. tenuis	+	−	−		
Moisture content 22.5% inoculated 4 hours after addition of sorbic					
A. niger	++		+	+	−
A. flavus	++++		++	++	−
A. ochraceus	++		−	−	−
A. candidus	++++		+	−	−
A. amstelodami	+++		++	−	−
P. chrysogenum	+		+	+	−
Alt. tenuis	+		+	−	−
Moisture content 29% inoculated one week after addition of sorbic					
A. niger	++		++	++	−
A. flavus	++++		+++	+	−
A. ochraceus	++		+	+	−
A. candidus	++++		+++	+	−
A. amstelodami	++		++	−	−
P. chrysogenum	++		+++	+++	−
Alt. tenuis	+		++	−	−

Lower concentrations of sorbic acid, especially in high moisture wheat, only retarded mold development on treated samples.

B. Inhibition of fungal growth on damp wheat.

Findings by visual inspection of wheat lots are summarized in Table 3. It is interesting that addition of sorbic acid, at the levels employed, prevented visible fungal growth and attack in lots kept at room temperature. On placing the samples at an elevated temperature, samples damped to 25% moisture showed signs of fungal attack. Practically all samples to which sorbic acid was added were characterized by germ discoloration.

Analyses of wheat and milling characteristics. Data on the biological and chemical effects of the treatment listed in Table 3 show that at the end of storage, samples treated with sorbic acid showed invariably a lower mold count and exhibited lower fat acidity values, as compared with corresponding untreated samples. The treatment not only retarded development of mold growth but also affected a relatively marked decrease in the number of viable molds in samples II, IV and V.

TABLE 3

Influence of moisture content and sorbic acid treatment on appearance, mold count, fat acidity and germination of wheat

No.	Grain treatment			Appearance after storage			Mold count	Fat acidity[1]	Germination
	Moisture	Sorbic acid		3-7 days	5 weeks (room temp.)	Additional week (30-32° C.)			
		Solid	Solution						
	%	%	%				*colonies/g*		%
I	12.5	.....	.....	sound	sound	sound	4,800	27.3	78
II	12.5	0.015	.....	sound	sound	sound	300	29.7	68
III	15.0	.....	.....	sound	sound	sound	3,300	39.8	73
IV	15.0	0.015	.....	sound	sound	sound	600	26.8	75
V	15.0	.....	0.015	small amount germ discoloration	small amount germ discoloration	small amount germ discoloration	600	25.9	51
VI	18.0	.....	.....	signs of fungal attack	advanced fungal attack	advanced fungal attack	6,400,000	70.5	7
VII	18.0	0.04	.....	sound	sound	fungal attack at germ tips	850,000	62.1	11
VIII	18.0	.....	0.04	germ discoloration	germ discoloration	germ discoloration	200,000	27.5	6
IX	20.0	.....	0.04	germ discoloration	germ discoloration	fungal attack at germ tips	500,000	60.1	15
X	25.0	.....	.....	advanced fungal attack	strong fungal attack	very strong fungal attack	10,500,000	179.5	2
XI	25.0	0.07	.....	very slight fungal attack	advanced fungal attack	very strong fungal attack	4,900,000	164.0	6
XII	25.0	.....	0.07	slight fungal attack	advanced fungal attack	advanced fungal attack	500,000	101.7	5
XIII	25.0	.....	0.10	germ discoloration	germ discoloration	fungal attack at germ tips	300,000	51.1	7

[1] mg KOH/100 g. wheat — dry matter basis.

Addition of sorbic acid had a profound effect on germination and even samples which showed no increase in mold count were characterized by decreased viability due to the action of sorbic acid. Adding sorbic acid in the form of powder was far less effective in checking deterioration as compared with liquid addition. The difference was caused, probably, by an insufficiently uniform distribution of sorbic acid added as a solid.

Results of milling tests show that addition of sorbic acid prevented increase in total flour yield as well as in break flour (expressed as percentage of total flour) features characteristic of wheat damaged by fungal attack *(10)*.

Changes in flour composition. The increase in flour extraction (shown in Table 4) was reflected in higher ash figures, poorer color (high color grade figures) and elevated diastatic activity. The addition of sorbic acid prevented these changes to a great extent.

TABLE 4

Influence of moisture content and sorbic acid treatment on milling characteristics and flour properties

No.	Grain treatment			Milling data		Flour quality		
	Moisture	Sorbic acid		Flour yield	Break flour	Ash[1]	Grade Color	Diastatic activity[2]
		Solid	Solution					
	%	%	%	%	*% of total flour*	%		
I	12.5	.....	.....	63.0	21.8	0.61	7.3	320
II	12.5	0.015	.....	62.3	23.4	0.56	7.7	310
III	15.0	.....	.....	61.7	22.0	0.54	6.8	300
IV	15.0	0.015	.....	60.1	24.0	0.53	6.5	290
V	15.0	.....	0.015	58.1	23.1	0.54	7.0	330
VI	18.0	.....	.....	63.9	22.7	0.57	9.2	340
VII	18.0	0.04	.....	61.9	24.9	0.59	8.7	290
VIII	18.0	.....	0.04	60.6	27.0	0.61	7.2	390
IX	20.0	.....	0.04	60.3	25.2	0.60	8.5	370
X	25.0	.....	.....	66.2	29.8	1.06	above 18	760
XI	25.0	0.07	.....	61.5	25.1	0.79	12.8	500
XII	25.0	.....	0.07	63.8	24.9	0.84	12.4	340
XIII	25.0	.....	0.10	62.7	25.3	0.75	9.8	315

[1] Calculated on 14% moisture basis.
[2] Calculated as mg maltose/10 g. of flour 14% moisture basis.

Dough characteristics and bread quality. Data on water absorption and dough characteristics (as determined on the farinograph) are summarized in Table 5. Flour milled from damp (18 and 25% moisture) wheat treated with sorbic acid showed higher water absorption than flour derived from untreated wheat. Regarding dough development, time and consistency drop, an improvement was observed only in flour milled from 25% moisture wheat.

No conclusive changes in extensibility and elasticity (as assessed with the extensograph) of doughs to which up to 0.2% sorbic acid (flour basis) was added in the form of powder, were observed.

TABLE 5

Influence of moisture content and sorbic acid treatment on dough characteristics and bread quality

Grain treatment				Dough characteristics				Bread quality		
No.	Moisture	Sorbic acid		Water absorption	Dough development time	Consistency drop after		Loaf volume	Crumb texture and appearance[1]	Taste and odor[1]
		Solid	Solution			15 min. mixing	3 hours rest			
	%	%	%	%	*minutes*	*B.U.*	*B.U.*	*cc*		
I	12.5	.....	.....	72.5	6.5	70	70	2050	open	.....
II	12.5	0.015	.....	73.1	6.0	100	120	1900	open	.....
III	15.0	.....	.....	71.1	7.0	70	100	2050	open	.....
IV	15.0	0.015	.....	71.0	7.0	80	110	2050	open	.....
V	15.0	.....	0.015	71.0	7.0	90	150	2000	open	.....
VI	18.0	.....	.....	64.3	7.0	60	80	1950	holes	.....
VII	18.0	0.04	.....	70.6	7.0	60	70	1950	open	.....
VIII	18.0	.....	0.04	69.4	7.5	80	100	1825	open	.....
IX	20.0	.....	0.04	73.8	7.5	90	80	1850	open	.....
X	25.0	.....	.....	58.5	1.0	300	390	1175	very dense, rubbery	bad, musty
XI	25.0	0.07	.....	72.0	1.0	140	250	1525	dense, rubbery	bad
XII	25.0	.....	0.07	64.5	6.0	120	280	1725	dense	bad
XIII	25.0	.....	0.10	64.0	7.0	100	260	1850	open	.....

[1] As compared with sample No. 1.

Results of loaf volume determinations and bread quality show effect of treatment on bread characteristics. In cases in which pronounced deterioration of dough characteristics, caused by mold growth on untreated wheat, was observed, the extent of deterioration was decreased in flour milled from grain samples, treated with sorbic acid. Whereas treatment with sorbic acid, per se, caused no practical changes in dough characteristics, a small decrease in loaf volume can be observed. But flour milled from treated 25% moisture wheat shows an improvement which outweighs by far the deteriorating effect of sorbic acid mentioned above. At the concentrations applied to wheat, sorbic acid seems to have little effect on the fermentation of dough and on loaf volume and does not affect grain, texture or taste of the loaf (Figures 1 and 2).

No study on the rate of penetration of sorbic acid into wheat was made. As the presence of sorbic acid in flour, at a level above 0.05%, was found to retard fermentation, it seems desirable to secure data regarding the movement of sorbic acid, applied on the surface, into the grain under various conditions of storage and wheat conditioning. Additionally, it would be desirable to learn about the influence of wheat cleaning procedures, i.e., scouring and washing as practiced industrially, on the extent of sorbic acid removal, especially when added in powder form.

The profound effect of sorbic acid on germination excludes the possibility of use of treated grain for malt production or seeding purposes.

Moldy grain is considered unsuited to the wet milling starch industry because of difficulty in separating the products and obtaining high yield and quality products *(6)*. As the treatment with sorbic acid was shown to prevent bran fracturing and flour contamination, the treated wheat may show industrial usefulness. Another aspect worth-while considering is the use of sorbic acid treated grain for short term storage of high moisture grain or for animal feed.

SUMMARY

Growth of seven species of mold on sterile wheat was affected by various levels of sorbic acid, depending on the moisture content of the damped wheat, and inhibited by treatment with 0.2% sodium sorbate.

Sorbic acid inhibits mold development on damp wheat in reasonable degree. When used at the proper level sorbic acid also inhibited to a large extent the growth of microorganisms. It enhanced the stability of the triglyceride fraction of the grain, and diminished the undesirable effect of fungal attack on milling characteristics. Treatment with sorbic acid reduces significantly seed viability.

Sorbic acid reduces loaf volume potentialities when added to normal flour at levels greater than about 0.05%, but this effect was considerably diminished when similar concentrations were applied to wheat. Nevertheless, this treatment inhibited losses in bread quality and dough fermentation characteristics caused by storage of damp grain.

Acknowledgment

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STERILIZATION BY MEANS OF SPORE DEACTIVATION[a]

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(Manuscript received July 3, 1957)

Curran and Evans *(1)* first reported the phenomenon of spore deactivation. This occurred when spore suspensions of a number of members of the genus *Bacillus* were preheated for activation and then stored under non-nutrient conditions. The result of deactivation is a marked drop in viable count during the storage period. In some cases a second preheating reactivated part or all of the apparently non-viable population. They found that spores surviving a process in whole milk and stored below growth temperature also exhibit the phenomenon of deactivation. Pearce and Wheaton *(2)* presented data upon the apparent loss of viability of spores of thermophilic flat sour organisms surviving experimental processes in cream style corn, peas, and dog food which occurred during storage at 70° F. for periods up to a year. This phenomenon was found not to take place with these products with spores of one thermophilic anaerobe under similar storage conditions. Pearce and Wheaton *(2)* use the term autosterilization to describe this process although pointing out that the usual meaning of autosterilization refers to the self-destruction of a culture due to conditions produced by its growth such as exhaustion of nutrients and toxic metabolic products. It appears to us that the best term to describe this behavior is *sterilization by means of spore deactivation*, since this is the basic phenomenon involved. This also permits discussion of deactivation which results in only partial sterilization of the units involved.

The potential usefulness of sterilization by means of spore deactivation in producing canned foods stable to thermophilic storage temperatures without excessive heat treatment assumes greater importance with the current interest in the canteen distribution of hot dispensed foods. Since these foods will be stored at thermophilic incubation temperatures, normally processed products may be susceptible to spoilage due to the surviving spores of thermophilic organisms. Due to the very high thermal resistance of the spores of thermophilic flat sour organisms the elimination of even moderate spore loads by thermal processing appears impractical. The data of Pearce and Wheaton *(2)* did not appear very encouraging for the achievement of sterilization by means of spore deactivation due to the prolonged storage period at 70° F. (4 to 8 months) required in order to prevent spoilage upon reincubation at thermophilic temperatures. Some observations made upon the effect of storage temperature on the rate of deactivation of thermophilic flat sour spores led us to a reinvestigation of this problem. This paper will present a part of our data on deactivation in nutrient media of mildly heated spores and the deactivation in cream style corn of the survivors of experimental processes.

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[a] Presented at the 17th Annual Meeting of the Institute of Food Technologists at Pittsburgh, Pennsylvania, May 14, 1957.

EXPERIMENTAL

Stock spore suspensions of the following 3 thermophilic flat sour organisms and one mesophile were used and may be identified as follows:

1518—N.C.A. 1518 strain
5341—Commercial spoilage in peas
617—Commercial spoilage in evaporated milk
5230—*Bacillus subtilis* 15 u from Dr. H. R. Curran

For deactivation tests in media the dilute suspensions in water were preheated for 15 minutes at 212° F. and plated on Difco Dextrose-Tryptone Agar containing 0.5% soluble starch. Control plates were incubated at 130° F. immediately; others were stored at various temperatures for deactivation and then incubated at 130° F. for residual count. Deactivation of the 5230 suspension under non-nutrient conditions was determined by preheating in water for 15 minutes at 212° F., plating immediately and plating following storage.

The deactivation tests in cream style corn were conducted by processing in T.D.T. cans, storing the cans at different temperatures then reincubating for 3 weeks at 130° F. and examining for spoilage. For each variable of time and temperature of storage 30 processed T.D.T. cans were used. The substrate was unprocessed cream style corn which had been held in the frozen state. The cans were inoculated and processed in the T.D.T. retort system as described by Schmidt, Bock and Moberg *(3)*. Since the suspensions had been standardized in cream style corn for D_{250} values, it was possible to calculate the theoretical number of survivors from the inoculum and process time.

RESULTS

Table 1 shows the deactivation which takes place under complete nutrient conditions with storage below minimum growth temperature. Since this table is compiled from different experiments, the initial count following heat activation shows some variation. The important point to be noted is the marked dependence of rate of deactivation upon temperature. For instance, after one day there were 70% survivors at 70° F., 52% at 85° F. and 33% at 98° F. It was the results of this type of experiment conducted with the different suspensions that suggested that storage temperature may have a profound effect upon the deactivation of surviving spores in a product.

Table 2 serves to illustrate deactivation under non-nutrient conditions using the mesophilic organism. It is apparent that deactivation takes place considerably more rapidly at 70° F. than at 60° F. and 50° F. In this particular instance the deactivation rate at 50 and 60° F. was the same.

Table 3 presents data on the effect of storage temperature on deactivation in cream style corn of surviving spores of 1518. At 70° F. storage the rate of deactivation is slow;

TABLE 1

Deactivation of spores of 1518 stored below growth temperature in an adequate growth medium

Initial count	Storage		Survivor count	% Survivors
	Time (days)	Temp. (° F.)		
201	1	50	200	100
208	1	70	139	70
273	2	70	66	24
204	14	70	13	6
265	1	85	139	52
265	2	85	42	16
265	11	85	12	5
208	1	98	66	33

TABLE 2

Deactivation of spores of 5230 in a non-nutrient substrate

Storage temp., ° F.	Mean plate count			
	Storage time in days			
	0	5	10	14
50	100	96	50	40
60	100	91	54	46
70	100	35	8	6

after 64 days more than 50% of the cans contain survivors. At 85° F. the rate of sterilization by deactivation is more rapid and at 98° F. complete sterilization of the 30 cans has been achieved in 14 days. Table 4 shows similar results with 617 suspension. It may be seen that all cans spoiled after 112 days at 70° F. with a low spore load. With a theoretical 10 or 100 spores per can following processing, sterilization by deactivation occurred after 21 days at either 85° or 98° F. but not after 14 days.

Table 5 presents some additional data upon the effect of residual spore load upon the rate of sterilization by deactivation. Although these tests were not carried to the stage of complete sterilization, they indicate the definite effect of residual level of spores following processing upon the time of storage which may be required to achieve sterilization.

Attempts were made to reactivate spores which had been stored sufficiently long in cream style corn to produce complete deactivation. Reactivation was attempted by heating such cans at 212° F. for 15 minutes just prior to incubation at 130° F. In all tests no evidence of reactivation has been found.

DISCUSSION

We define *spore deactivation* as the loss of ability to germinate and develop under favorable growth conditions acquired by a spore stored under conditions preventing vegetative development. As shown by Curran and Evans *(1)*, and as observed in our laboratory, under certain conditions some spores which have become deactivated may be reactivated; therefore, we may have either *reversible* or *irreversible deactivation.* Irreversible deactivation of all the spores in a unit container leads to sterilization. It may be that irreversible deactivation represents the first stages of germination which lead to loss of

TABLE 3

Effect of storage temperature upon the deactivation of spores of 1518 surviving in cream style corn processed at 250° F.

Inoculum per can	Process time minutes	Theoretical survivors/can	Storage		Survivors on incubation
			Time (days)	Temp. ° F.	
2000	20.0	10	32	70	30/30
2000	20.0	10	35	70	30/30
2000	20.0	10	64	70	17/30
2000	20.0	10	14	85	20/30
2000	20.0	10	21	85	1/30
2000	20.0	10	4	98	30/30
2000	20.0	10	7	98	12/30
2000	20.0	10	14	98	0/30

TABLE 4

Effect of storage temperature upon the deactivation of spores of 617 surviving in cream style corn processed at 250° F.

Inoculum per can	Process time minutes	Theoretical survivors/can	Storage		Survivors on incubation
			Time (days)	Temp. ° F.	
10,000	6.0	5	112	70	30/30
10,000	3.0	10	14	85	30/30
10,000	3.0	10	21	85	0/30
10,000	2.0	100	14	85	30/30
10,000	2.0	100	21	85	0/30
10,000	3.0	10	14	98	28/30
10,000	3.0	10	21	98	0/30
10,000	2.0	100	14	98	30/30
10,000	2.0	100	21	98	0/30

viability if all the conditions for vegetative growth are not available. This, however, has not as yet been conclusively demonstrated. Deactivations which are reversible under some conditions could still lead to sterilization if not reversible under conditions of thermophilic incubation.

Results presented in this paper confirm the fact that deactivation of either mildly heated spores or the survivors of a process can occur under conditions preventing vegetative development. They also emphasize the profound effect that storage temperature has upon the rate at which deactivation proceeds. We believe the results offer some hope that small residual spore loads surviving processing may be deactivated during a reasonable period of time by controlling the storage temperature in order to achieve sterilization by means of spore deactivation. Before attempts are made to exploit such a procedure more work is necessary with a larger and more representative number of strains of thermophilic flat sours. Also the deactivation of the spores should be studied in relation to the permissible levels of processing in any product that is of specific interest.

Although we have no data to present at this time, the discovery of the profound effect of temperature upon the rate of deactivation also provides

TABLE 5

Effect of survivor level upon the time required to produce sterile samples

Suspension	Theoretical survivors/can	Storage		Survivors on incubation
		Time (days)	Temp. ° F.	
1518	10	14	85	20/30
	100	14	85	30/30
	10	21	85	1/30
	100	21	85	11/30
5341	10	14	98	16/30
	100	14	98	30/30

some hope that conditions may be found to produce deactivation of spores of the thermophilic anaerobes. Also, although it would be premature at this time to name specific substances, we have some indications that certain substances whose addition to a food product would be permissible may accelerate the rate of deactivation.

Since deactivation of mildly heated thermophilic spores proceeds in a complete nutrient medium or in water below growth temperatures, readily usable experimental procedures are available by which to study the effect of specific nutrient conditions or activators and inhibitors on the rate of deactivation.

SUMMARY

The main previously known facts about spore deactivation have been confirmed. In addition it has been demonstrated that temperature of storage has a profound influence on the rate of deactivation of spores of several thermophilic strains either in nutrient culture media with mildly heated spores or in cream style corn with survivors of a process. Spores of one strain of a mesophile show deactivation following preheating in water and the rate is somewhat temperature dependent. The results offer some hope that small residual spore loads surviving processing may be deactivated during a reasonable period of storage by controlling the storage temperature in order to achieve sterilization by means of spore deactivation.

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MAPLE SIRUP. XI. RELATIONSHIP BETWEEN THE TYPE AND ORIGIN OF REDUCING SUGARS IN SAP AND THE COLOR AND FLAVOR OF MAPLE SIRUP[a]

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Maple sirup made from early, cold-weather sap flows or from sterile sap has a light amber color and a delicate flavor. Sirup made from late-season or from fermented sap usually possesses a flavor denoted as caramel and also has a dark amber or brown color which decreases its value *(2, 3, 6, 7)*. Analyses of sirups have shown that with increasing coloration and caramel flavor there is also an increase in concentration of reducing sugars (invert) *(2, 10)*. Fresh, sterile maple sap contains no hexose sugars *(4, 8)*, but fermentation of sap with certain microorganisms results in sirup containing invert sugar and caramel *(5)*. It has also been shown, however, that under certain controlled conditions, fermentation of sap results in enhancement of the maple flavor without the production of caramel.

This paper reports studies made to determine the effect of the type and origin of reducing sugars in sap on the formation of color and flavor in maple sirup. To determine their possible specificity in producing these color and flavor changes, the following reducing sugars were added to sterile sap before concentrating it to sirup: simulated invert obtained from a mixture of crystalline glucose and fructose; crystalline maltose; reducing sugars derived from crystalline sucrose and maltose by enzymic and ion-exchange resin hydrolysis; and reducing sugars from carbohydrates in sap by the action of invertase, which was added to the sterile sap 4 days before concentrating it to sirup.

MATERIALS, METHODS, AND PROCEDURE

Materials. Maple sap was collected under aseptic conditions using the technique previously described *(7)*. The sap from each sterile 5-gallon collecting bottle was aseptically dispensed into sterile 1-gallon metal cans, quick-frozen, and stored at −29° C. The sterility of the collected sap was determined by sampling each collecting bottle and plating the samples in nutrient agar. Any lot of sap having a count exceeding 1 colony per milliliter was discarded. Since only 4 portions of sap could be conveniently reduced to sirup in 1 day, the experiment was carried out at 4 different times with 4 lots of sap. Careful selection of sap was required so that identical replicates could be used throughout the 4 parts of the study. To obtain this uniformity, eight 4-gallon collections of sterile sap were selected. One gallon of sap from each collection was allocated to each of the 4 lots so that at the completion of the allocation each 8-gallon lot contained equal volumes of sap from the same trees and the same flows.

To prepare each lot of frozen sap for use, a procedure was developed to thaw the sap rapidly in a manner which minimized microbial contamination and chemical changes. The desired amount of frozen sap was removed from the gallon cans, crushed in a

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sanitized power crusher and melted rapidly in a steam-jacketed kettle. To prevent changes in the sap during heating, the kettle was charged with about 1 gallon of the crushed frozen sap, heat was applied by steam at 5 p.s.i., and the ice was slurried rapidly until most of it had melted, at which time the sap was removed. In a short time the residual ice melted, the final temperature of the sap being 15°–20° C. The melted sap was blended and divided into 4 portions of 2 gallons each.

The carbohydrates were added to the sap at a concentration of 1 g. per 2 gal. The reducing sugars evaluated in this study consisted of the following:

a. Simulated invert was prepared by combining equal weights of crystalline glucose and fructose.
b. Invert sugar was prepared from sucrose by 3 methods. These methods of inverting sucrose were investigated to determine if they produced cleavage products with different or peculiar reactivities, even though of a transitory nature.
   1. Enzyme inversion. A 10% solution of sucrose was treated with invertase (0.2 ml. of Convertit [c] per 10 ml. of solution) for 2 hours at 15° C.
   2. Ion-exchange inversion. A 4% solution of sucrose was treated with Dowex-50 resin [c] in the H-ion phase. Fifty milliliters of sucrose solution was heated with 10 g. of Dowex-50 for 10 min. at 65–70° C. The mixture was filtered to remove the resin and washed to a volume of 100 ml. A 50-ml. aliquot (equivalent to 1 g. of carbohydrate) was added to the sap immediately before reduction to sirup.
   3. Inversion of sucrose of maple sap. A 2-gallon portion of sap was treated with 0.2 ml. of Convertit [c] and incubated for 4 days at about 1° C.
c. Maltose was used as a representative reducing disaccharide.
d. Hydrolyzed maltose was prepared by treating with Dowex-50 resin in the H-ion phase. Fifty milliliters of a 4% solution of maltose was treated with 10 g. of Dowex-50 for 2 hours at 65–70° C. The mixture was filtered to remove the resin, washed to 100 ml. and a 50-ml. aliquot was added to the sap immediately before reduction to sirup.

Methods. Refractive index measurements for determining per cent solids as sucrose (degrees Brix) were made at 20° C. with an Abbe refractometer. Color indices [d] were

[c] Mention of trade name does not imply endorsement of the U. S. Department of Agriculture over similar products not mentioned.

[d] Color index, $A_{1\ cm.}^{86.3\%} = A_{450}\ (86.3/bc)$, where A_{450} is the observed absorbance at 450 mμ, b is the depth of solution in centimeters and c is the grams of solids as sucrose per 100 ml.

TABLE 1

Arrangement of experimental treatments and controls among the four lots of sap

Portion	Lot A	Lot B	Lot C	Lot D
1	Untreated control	Sucrose	With invertase for 4 days[1] at about 1° C.	Maltose hydrolyzed with Dowex-50
2	Sucrose inverted with invertase	Untreated control	Simulated invert	Sucrose inverted with invertase
3	Inactivated invertase	Maltose	Untreated control	With invertase for 4 days[1] at about 1° C.
4	Simulated invert	Sucrose inverted with Dowex-50	Sucrose inverted with invertase	Untreated control

[1] The sap in this instance was reduced to sirup 4 days later than other portions from this lot.

determined from absorption measurements at 450 mμ made on sirups of 65-67° Brix filtered with pressure through three layers of Whatman ® No. 52 filter paper in a Seitz filter. Per cent of invert sugar was determined by the Berlin method *(1)*. Flavor evaluations of the sirups were made by an experienced panel.

Procedure. An 8-gallon lot of frozen sap was thawed for each of the 4 parts of the study. The melted sap was divided into 2-gallon portions and treated as shown in Table 1. This randomized distribution was selected to avoid any differences that might have been caused by variations in daily or within-day operations. One portion from each lot was an untreated control, while reagent controls were provided by one portion that was treated with 1 g. of sucrose (B1) and one with 0.2 ml. of inactivated invertase (A3).

The 2-gallon portions of sap were reduced to sirup under standardized conditions selected to simulate conditions occurring in a commercial evaporator. In a steam-jacketed kettle, sap was evaporated rapidly (30–35 minutes) to a volume calculated to give a density of approximately 45° Brix. This sirup was transferred to a stainless steel beaker equipped with a condenser and refluxed for one hour over a Meeker burner. The refluxed sirup was transferred to a 1-quart steam kettle and evaporated rapidly (5–8 minutes) to standard density sirup (65.5° Brix).

Determinations were made on the sirups prepared from treated sap as follows: per cent of invert sugar, color indices, and flavor evaluations.

RESULTS AND DISCUSSION

Effects of adding reducing sugars to maple sap on the color and flavor of the sirup are summarized in Table 2. The close agreement in values obtained for reducing sugars calculated as invert sugar and for color index in both the untreated and reagent control samples indicates that the empirical procedure devised is reproducible from day to day and that addition of the reagents did not affect the flavor, color, or invert content of the sirup produced.

Adding reducing sugars derived from sucrose either by enzyme inversion, by ion-exchange hydrolysis, or by simulation with equal parts of glucose and

TABLE 2

Effect of type and source of reducing sugar on color and flavor of maple sirup

Lot—Portion	Treatment	Invert sugar	Color index	Degree of caramel[1]
		%		
A1	Untreated control	0.15	0.51	– – – –
B2	Untreated control	0.16	0.51	– – – –
C3	Untreated control	0.16	0.52	– – – –
D4	Untreated control	0.18	0.56	– – – –
B1	Sucrose	0.16	0.51	– – – –
A3	Inactivated invertase	0.16	0.50	– – – –
C2	Simulated invert	0.55	0.75	+
A4	Simulated invert	0.81	0.92	++
A2	Sucrose inverted with invertase	0.72	0.91	++
C4	Sucrose inverted with invertase	0.80	0.77	++
D2	Sucrose inverted with invertase	0.74	0.98	++
B4	Sucrose inverted with Dowex-50	0.41	0.72	++
C1	Sap treated with invertase for 4 days at 33-34° F.	53.3	3.25	++++
D3	Sap treated with invertase for 4 days at 33-34° F.	56.3	2.90	++++
B3	Maltose	0.56	0.77	++
D1	Maltose hydrolyzed with Dowex-50	0.49	0.87	+

[1] Indicates no caramel detected; + to ++++ indicates increasing degrees of caramel.

fructose resulted in the production of appreciable color and caramel flavor.* When sap was treated with invertase for 4 days at about 1° C., resulting sirup had an exceedingly dark color and strong caramel flavor. Under the conditions used over half of the sucrose present was inverted.

Maltose was chosen as an example of a reducing oligosaccharide which upon hydrolysis would yield glucose only. Use of this carbohydrate and its hydrolytic products also resulted in increased color and the production of caramel flavor.

Addition of reducing sugars did not seem to increase the level of maple flavor, although it is quite possible that the presence of caramel obscured any maple flavor that might have been formed. Development of color and caramel flavor from sap containing invert sugar is consistent with facts observed under practical conditions of maple sirup production. In most cases the darker grades of sirup are characterized by stronger caramel flavor and an increasing content of invert sugar *(2, 10)*. Such sirups are produced under conditions favoring microbial growth, and the reducing sugars must be the product of sucrose inversion. It has been shown that sterile sap contains no measurable amount of hexoses and only a trace of oligosaccharides which could be reducing *(8)*.

Recently, Underwood, Lento and Willits *(9)* have demonstrated the presence of trioses in maple sirup. This offers a possible mechanism whereby reducing sugars give rise to the development of color and flavor. However, this does not fully explain the enhancement of maple flavor following the controlled fermentation of sap with a *Pseudomonas sp. (5)*. The possible role of other products of the fermentation is strongly suggested.

* This is the sharp pungent flavor so characteristic of dark sirups.

SUMMARY

Incorporation of reducing sugars in maple sap before reduction to sirup by atmospheric boiling results in increased color and caramel flavor irrespective of the source of the sugars. Although there was no detectable enhancement of maple flavor, this could have been obscured by the presence of caramel.

Acknowledgment

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USE OF HIGH-VOLTAGE CATHODE RAYS TO DESTROY BACTERIA OF THE *SALMONELLA* GROUP IN WHOLE EGG SOLIDS, EGG YOLK SOLIDS, AND FROZEN EGG YOLK [a, b, c]

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Presence of bacteria of the *Salmonella* group in egg products and other foods is known to constitute an important health hazard *(3)*. Although the counts reported have generally been low *(2)*, Byrne and Rayman *(1)* found that liquid egg, before drying, might contain 1,000 to 30,000 *Salmonella* per gram of solids.

Many studies have been made to determine methods of eliminating *Salmonella* from egg products, particularly by pasteurization *(12)*. However, egg products are relatively heat sensitive and cannot be heated at temperatures sufficiently high to ensure the complete destruction of large numbers of *Salmonella* without exhibiting changes in functional properties. Whole egg and yolk are frequently subjected to a pasteurizing treatment of 2 minutes or more at temperatures near 140° F. The lack of a completely satisfactory means of destroying *Salmonella* in egg products by heating has added impetus to the search for alternate destruction methods such as the use of "cold" high energy radiation.

Research has been carried out for several years to determine the efficacy of high-voltage cathode rays, produced by a Van de Graaf electrostatic generator, for the pasteurization and sterilization of food products. Early studies at the Massachusetts Institute of Technology *(8)* indicated that relatively low doses were required to destroy *Salmonella* in liquid whole egg. However, some flavor changes were noted in scrambled eggs prepared from the irradiated material. Spray drying of the liquid product after irradiation resulted in the elimination of any significant flavor difference.

This preliminary investigation led to the present study. Results relating to various egg white products have already been reported *(5)* and have shown

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that an irradiation dose that caused a 10^7 fold reduction in *Salmonella* organisms in egg white products caused only minor deteriorations in their functional and organoleptic properties.

The present paper is concerned with the determinations of the doses of high-voltage cathode rays necessary to destroy *Salmonella* in whole egg solids, egg yolk solids and in frozen egg yolk, and with the effect of high-voltage cathode rays on the flavor and functional qualities of these products. Investigations concerning possible means of alleviating irradiation-induced off-flavors in the products studied are also reported.

MATERIALS AND METHODS

Preparation of egg products. Egg solids and frozen egg yolk used in this investigation were obtained from commercial manufacturers. Whole egg solids and egg yolk solids were commercially spray dried after being processed with glucose-oxidase enzyme to remove glucose. Free glucose in the dried product was reduced to a level of 0.05% or less. The liquid products after being processed to remove the reducing sugars, were flash pasteurized at a temperature of 142° F. and held at this temperature for 2.5 minutes before being spray dried. The frozen egg yolk contained 10% added sucrose. All egg products were stored at 0° F. until used.

Spray drying and lyophilization of irradiated and unirradiated fresh egg products for organoleptic evaluations were carried out as follows: spray drying of whole egg and egg yolk which had been previously irradiated with 850,000 rep was carried out in a laboratory model spray drier consisting of a conical chamber in which heated air, admitted concurrently with the sprayed egg product, was dispersed by the use of a rapidly rotating disk. This spray drying was accomplished with an inlet air temperature of 300° F., and an outlet air temperature of 200° F.

Lyophilization of irradiated and unirradiated whole egg and egg yolk was accomplished by freezing in a mixture of dry ice and alcohol and drying overnight in a lyophilizer under high vacuum.

Bacteriological techniques. The bacteriological techniques used for the preparation of inoculum, preparation of samples containing *Salmonella* organisms for irradiation, the culturing of irradiated samples, the plate count method for determining *Salmonella*, and the determination of sterilizing dose for uninoculated egg products have been previously described *(5)*. Briefly, the method used was as follows: *S. typhimurium* (ATCC 9187) and *S. senftenberg* (ATCC 775w) were used in experiments to determine the cathode ray radiation levels necessary to destroy *Salmonella* organisms in egg products. Suspensions of the organisms to be used in inoculating the egg products for irradiation with high-voltage cathode rays were prepared by scraping the colonies of these organisms from the surface of 72 hour bismuth sulfite agar plates. A portion of this inoculum was added to the egg product to be tested in such proportion as to bring the *Salmonella* count to between 5×10^5 and 5×10^7 organisms per gram. The inoculum was thoroughly mixed with the product.

Two-gram portions of inoculated product were weighed into 305 x 007 aluminum cans and sealed in an atmosphere either of air, oxygen, or nitrogen. After the irradiation treatment, approximately 10 ml. of selenite broth were added to each sample and the samples were incubated for 48 to 120 hours at 37° C. Variations in incubation time within this range did not not affect the results. After incubation, the material was streaked on bismuth sulfite agar which was incubated for 48 hours at 37° C., and then inspected for growth of typical colonies. These colonies were then confirmed by anti-serum agglutination tests. Frozen egg yolk was incubated for 48 to 120 hours at 37° C. before selenite broth was added, as this procedure had been found previously to increase the number of surviving microorganisms in this product. The samples were then reincubated for 48–120 hours before streaking on bismuth sulfite plates.

Egg products previously sterilized by exposure to a dose of 3×10^6 rep in a Van de Graaff generator were inoculated with *Salmonella* to give test materials having counts in excess of one million organisms per gram. Samples of the inoculated test material were irradiated at different dose levels and standard plate counts on nutrient agar determined.

In determining sterilizing doses for whole egg solids and for egg yolk solids, two types of containers were used. The first of these was made of pieces of tygon tubing, sealed at each end with one end of a gelatin capsule, the second of a soluble packaging film (polyvinyl alcohol). In each case the irradiated sample was transferred aseptically to 25 x 200 mm. culture tubes which contained 25 ml. of sterile thioglycollate broth. After being incubated for 14 days at 30° C. transfers were made to 18 x 150 mm. culture tubes that contained thioglycollate broth. Cultures in the small tubes were incubated for 2 to 7 days at 30° C. and any tubes showing growth after incubation were recorded as positive. Two-gram samples of frozen egg yolk were irradiated in small, shallow, aluminum containers. After irradiation, the samples were cultured in the manner used for egg solids.

Irradiation technique. Source of the cathode rays was a Van de Graaff accelerator with a 3 m.e.v. capacity. Samples of inoculated egg products in aluminum cans were placed on a motor driven belt, the path of which was traversed by the electron beam. Cans containing the samples were irradiated upside down to eliminate any possibility of the can rim interfering with penetration of the cathode rays. Cans containing frozen egg yolk were placed in a tray of dry ice during irradiation treatment. Samples used in the functional tests (uninoculated) were placed in either sardine cans or polyethylene bags for irradiation treatment.

Dosimetry. Ferrous dosimetry was used to measure the irradiation doses. In all cases a test was considered to be satisfactory if the requested dose differed from the calculated dose by not more than 10% *(11)*.

Fluorescence. Whole egg solids were defatted before fluorescence tests were conducted. This was done by mixing 2.5 g. of egg solids with 3 successive 25 ml. portions of chloroform. Exactly 1.0 g. of solvent-free defatted solids was used for fluorescence determinations which were carried out according to the method of Pearce, Thistle and Reid *(6)*. In each case the result given is the average of a duplicate analysis of a single sample.

Peroxide number. Irradiated and unirradiated whole egg solids, egg yolk solids, and frozen egg yolk were extracted with ethyl ether. The ether was evaporated to yield approximately 1 g. of oil. The sample of oil was weighed into a 2–3 ml. beaker and 1 ml. of saturated potassium iodide solution (approximately 150 g. of KI in 100 ml. of water) was added to another small beaker. The 2 beakers were placed upright in a 250 ml. Erlenmeyer flask containing 25 ml. of a 60/40 mixture of glacial acetic acid and ethylene dichloride. A two-hole rubber stopper was inserted tightly in the neck of the flask and a glass tube, connected to a carbon dioxide generator (dry ice), was inserted in the solvent for 10 to 15 minutes so that the oxygen might be eliminated. The glass tube was then removed and the holes in the stopper plugged immediately with short glass rods. The 3 components in the flask were mixed well by swirling the contents. After a 2-hour reaction time in the dark at room temperature, 50 ml. of boiled, cooled water were added and the mixture titrated with N/100 sodium thiosulfate solution. Starch was used as an indicator. The results are an average of a duplicate analysis of a single sample.

The peroxide values were calculated as millimoles of peroxide per 100 grams of oil:

$$\text{peroxide value} = \frac{\text{ml}_{\text{Na}_2\text{S}_2\text{O}_3} \times 0.01 \times 0.5 \times 1000}{\text{weight of sample}}$$

Whipping tests. A Bostick Consistometer was used to evaluate the whipping ability of unirradiated and irradiated whole egg solids, egg yolk solids, and frozen egg yolk.

Sixty grams of reconstituted whole egg solids, reconstituted egg yolk solids, or thawed frozen egg yolk were placed in the bowl of a Hobart Model K-4-B mixer. The mixer and bowl were placed in a water bath at 140° F. and the reconstituted whole egg or egg yolk whipped for varying periods at speed No. 10. Thawed frozen egg yolk was whipped at room temperature. The chamber of the Bostwick Consistometer was then filled with the whipped material and the lever was tripped. Readings of the distance of flow after various time intervals were plotted. From the resultant curves, the reciprocal of the distance flowed in 60 seconds was obtained. The specific gravity of the whipped material was also recorded in each case. The results reported were an average of several runs made on different batches of test material.

Cake volume. Sponge cakes made with samples of unirradiated and irradiated whole egg solids, egg yolk solids, and frozen egg yolk were used in these experiments. The cakes were baked according to the procedure of Kline *et al. (4)* with the exception that the hot water bath for whipping reconstituted whole egg solids and reconstituted egg yolk solids was maintained at 160° F. instead of 122° F. Thawed frozen egg yolk was whipped at room temperature. Various whipping times were used. The dried egg products were reconstituted as described by Kline *et al.*: a 2:1 ratio of water to dried material was used in the case of dried egg yolk.

Volumes of the duplicate cakes were determined by the rape seed displacement method. Several comparisons were made at each whipping time on different batches of material and the results averaged.

Organoleptic tests. Sponge cakes, custards, and scrambled eggs were subjected to a triangle-type test and also rated for acceptability according to scoring procedures in which numbers from 1 (dislike extremely) to 9 (like extremely) were assigned. Approximately 20 judges participated in each organoleptic test. In this test each judge was given 3 samples, 2 of which were the same and one of which was different and was asked to identify the like samples and to assign a flavor score to the item after segregation. The results of both the identification and the rating tests were subjected to statistical analysis by the "t" method of ascertaining significance. No order of tasting was specified to the judges and no more than two sets of samples were tested at one sitting. When calculating the hedonic numerical rating of each sample, and its significance, only the scores of those judges who correctly segregated the samples, or who assigned the same score to all samples were used.

Custards were baked according to the procedure cited in *Technical Bulletin 64*, Kansas State Agricultural Experiment Station *(10)*. Scrambled eggs were prepared from irradiated and unirradiated whole egg solids by reconstituting at a ratio of three parts water to one part whole egg solids, and scrambling in a double boiler. No spices or other flavoring agents were used in the preparation of test materials for organoleptic evaluation.

All organoleptic evaluations of these products were carried out under red light to minimize color difference. The results of organoleptic analyses are reported for each individual comparison. However, in most cases the several analyses reported for each product were made on different batches of egg product. Each time an organoleptic comparison was made, a new test material (e.g. sponge cake, custard, or scrambled egg) was used.

RESULTS AND DISCUSSION

Cathode ray lability of two Salmonella species. In determining the lability of *Salmonella* to cathode rays, two factors were determined: the specific inactivation dose. D_o values (dose required to destroy 62.5% of the organisms); and the destruction dose (dose required to cause 10^7-fold reduction in *Salmonella* count). Specific inactivation doses were obtained in two ways: (a) by determining D_o from ordinary survival curves usually covering the range of 50% to 1% survival and (b) by determining D_o from the number of 2-gram samples out of 5 showing growth at various dose levels, estimated by the method of Schmidt *(9)*. Determinations of destruction doses also were obtained in two ways: (a) by direct determination and (b) by calculation from the survival curve D_o.

In the Schmidt method, where none of the 5 samples shows growth, the most probable number of survivors is less than 0.1 per gram. Since the initial counts varied from 0.1 to 30 x 10^6, the latter determination is based on the survival of only about 0.00001% of the initial inoculum, and thus is about a 10^7 fold reduction in numbers. D_o values determined by this method were generally based on irradiation at 4 or more dosage levels. The specific inactivation doses determined in the two ways agreed within 35% of the mean of the two values (Table 1).

The D_o values estimated from the 10^7 fold destruction data tend to be smaller than the D_o values obtained from survival curves (Table 1). In the previous paper *(5)* concerning egg white products, a similar result was obtained. Results in both cases indicate that the target theory expression, $n/n_o = e^{-D/D_o}$, is a good representation of the irradiation destruction of *Salmonella* over a 10^7-fold destruction range. In this expression n_o is the initial number of microorganisms, n is the number surviving dose D, and D_o is the specific inactivation dose.

As noted previously *(5)*, it was found that the *Salmonella* destruction doses were essentially the same regardless of the atmosphere of irradiation

TABLE 1

Comparison of D_o values determined by two experimental procedures and of dose required to reduce Salmonella count approximately 10^7-fold determined experimentally and by calculation from survival curve D_o values [1]

Organism	Atmosphere	Specific inactivation dose, D_o		Initial *Salmonella* count (millions per gram)	Dose required to reduce count to approx. 1 per 10 gm.	
		From 10^7-fold reduction test (thousand rep)	From survival curve (thousand rep)		Calculated from survival D_o (thousand rep)	Directly determined values[1] (thousand rep)
Whole egg solids						
S. typhim.	Air	26 ± 21[2]	27 ± 10[2]	0.11–3.2	380–470	360–450
	Nitrogen	42	26 ± 7	2.9	450	720
	Oxygen	20 ± 9	25 ± 10	0.11–3.2	350–430	280–350
S. senft.	Air	17	22 ± 6	4.5	340	260
	Nitrogen	62	30 ± 3	0.45	460	950
	Oxygen	15 ± 6	20 ± 5	0.45–0.63	310–320	230–240
Egg yolk solids						
S. typhim.	Air	36	31 ± 18	2.4	530	620
	Nitrogen	38	43 ± 22	1.5	720	620
	Oxygen	48	31 ± 7	2.4	530	820
S. senft.	Air	39	53 ± 25	1.1	860	630
	Nitrogen	51	59 ± 81	1.1	950	830
	Oxygen	37 ± 17	50 ± 21	0.23–1.2	730–810	540–600
Frozen egg yolk						
S. typhim.	Air	25 ± 9	27 ± 5	0.74–15.0	430–520	390–460
	Nitrogen	23 ± 2	31 ± 7	6.9 –15.0	560–590	420–440
	Oxygen	19 ± 14	26 ± 10	0.50–15.0	400–500	290–360
S. senft.	Air	18 ± 7	25 ± 2	0.56–30.0	390–490	280–360
	Nitrogen	23 ± 20	27 ± 8	0.56–30.0	420–530	360–450
	Oxygen	18 ± 11	27 ± 25	0.56–30.0	430–500	280–350

[1] These values were calculated from the number of tubes out of five (each containing two grams) showing growth at various dose levels by the method of Schmidt *(9)*. The values for D_o in column three are essentially extrapolated values from these data. The values in columns six and seven are influenced by the initial count.

[2] Mean value with confidence limits (standard error x t).

(Table 1). Therefore, in comparing the effects of other treatments it is justified and helpful to consider the runs in the various atmospheres as simple replicates, and Table 2 is derived in this manner.

In Table 2, average destruction doses derived by the two methods (direct determination and calculation from D_o) are shown. In all cases, average doses are nearly the same regardless of the method used to determine the values.

In the research carried out with egg white products *(5)*, it was found that 2 to 3 times the dose was required to kill *Salmonella* in egg white solids than in frozen or liquid egg white. Comparison of the data in Table 2 with the previous results show that the destruction dose (10^7-fold) required for *Salmonella* in egg yolk solids is nearly the same as that found in egg white solids (unsugared), whereas the dose required in whole egg solids was slightly lower. The dose required for frozen egg yolk was nearly the same as that required for whole egg solids, but was significantly higher than the dose required for either frozen or liquid egg white.

Data given in Table 2 indicate that the lability of *S. senftenberg* and *S. typhimurium* is nearly the same when any one of the egg products (whole egg solids, egg yolk solids or frozen egg yolk) is considered. Although variations occurred there is no consistent difference in sensitivity between the two species in the products tested.

Consideration of "practical" destruction dose evaluation. In the previous paper concerning egg white products, a discussion of the considerations given in determining a practical destruction dose were outlined. In this discussion it was pointed out that a 10^{11} fold reduction would result in a reduction from an initial contamination of 100 *Salmonella* per gram to one viable *Salmonella* per 10^9 grams or 2.2 million pounds of egg solids.

TABLE 2

Comparison of average destruction doses obtained for three types of egg and two Salmonella species [1]

Type of egg	*Salmonella* species	Destruction dose			
		10^7-fold kill		10^{11}-fold kill[3]	
		from 10^7-fold kill data[2] (thousand rep)	from survival curve (thousand rep)	from 10^7-fold kill data (thousand rep)	from survival curve (thousand rep)
Whole egg solids	*typhim.*	470	420	740	660
Whole egg solids	*senft.*	510	380	790	610
Egg yolk solids	*typhim.*	640	560	1,030	890
Egg yolk solids	*senft.*	680	870	1,070	1,370
Frozen egg yolk	*typhim.*	360	450	570	710
Frozen egg yolk	*senft.*	320	410	500	650

[1] The values reported in each case are averages of data obtained in air, nitrogen, and oxygen, since these treatments did not influence the dose requirements under the conditions used in this study.
[2] These values are for practical purposes observed values, since they were obtained by the method of Schmidt *(9)* from actual kills of this order of magnitude corrected only for the relatively minor differences in initial counts.
[3] These are extrapolated values.

TABLE 3

Effect of high-voltage cathode rays on growth of organisms of all types in uninoculated egg products[1]

Egg product	Type of container	Original number of organisms (per gram)	Sterility dose (thousand rep)
Whole egg solids	Tygon tubing	94,000	1,500–3,500
	Reynolon 4801	34,000–81,000	1,600–3,500
Egg yolk solids	Tygon tubing	4,600	1,500–2,500
	Reynolon 4801	7,400–13,000	1,400–3,500
Frozen egg yolk	Aluminum "boats"	220,000	700–1,000

[1] These tests were made to determine the lethality of cathode rays on all organisms (including spore formers) normally found in the egg products treated.

Table 2 includes the dose required for such a destruction. This was calculated from the equation:

$$D = 2.3D_a a$$

where the dose D corresponds to the factor a and a is the reduction factor required (in this case, 11).

Destruction of microorganisms other than Salmonella. Two- to five-fold higher doses were required to destroy the various contaminating organisms found in uninoculated egg products than were required for the destruction of *Salmonella* (Table 3).

Organoleptic and chemical tests and cake-mixing properties of egg yolk and whole egg treated with cathode rays. Triangle difference tests indicated that in nearly all cases the irradiation of whole egg solids to be incorporated in sponge cakes, custards and scrambled eggs had a significant effect on flavor preference (Table 4). In general, for sponge cakes the significance level was sufficiently low to suggest that the flavor changes were relatively small. However, with custards and scrambled eggs all but one of the flavor differences were significant at a high level (0.1 to 0.001%) and the products were distinctly affected.

Egg yolk solids were evaluated organoleptically under red light after being incorporated in sponge cakes and custards. It would appear that irradiation of egg yolk solids at a dose level of 850,000 rep produced only minor flavor changes in sponge cakes made with the egg product (Table 5). However, in the case of custards, the results showed that irradiation usually caused a highly significant flavor change.

The flavor difference between sponge cakes prepared from irradiated (600,000 rep) and unirradiated frozen egg yolk was slight, but a significant preference at a high level (1 to 0.01%) was shown in most instances for custards prepared from the unirradiated material (Table 6).

There was a tendency for the irradiated egg yolk solid samples to require a longer whipping time to obtain maximum sponge cake volumes, but it is considered that this effect is not of great importance (Figure 1).

For whole egg solids the volumes of sponge cakes prepared from the irradiated material were approximately 20% less than those prepared from

unirradiated whole egg solids. The two materials responded differently to whipping times, as is shown in Figure 2.

There was little difference in volumes of sponge cakes prepared from irradiated and unirradiated frozen egg yolk (Table 7). It would appear that in both cases maximum cake volumes were achieved by whipping for 4 to 5 minutes.

A consistency measurement, based on a method developed in connection with this investigation, was used as an empirical measurement of the effect of cathode ray radiation on egg products. So far as is known there is no relationship between consistency and performance in egg products of this kind. Egg yolk and whole egg solids are ordinarily used in the preparation of foods other than sponge cakes, but sponge cakes were used to evaluate the quality of irradiated and unirradiated egg products because this test was considered to be more sensitive to change than other available tests.

Irradiation of frozen egg yolk at 850,000 rep had little or no effect on either the consistency or the specific gravity of whipped material (Table 8).

Both irradiated (850,000 rep) and unirradiated egg yolk solids (Figure 3) showed a similar variation of consistency with whipping time. In both cases, as whipping time increased, the consistency increased; after the maximum was reached the consistency decreased with increased whipping time. How-

TABLE 4

Acceptability scores for sponge cakes, custards, and scrambled eggs made with irradiated (850,000 rep) and unirradiated commercially dried whole egg solids

Sample	Acceptability scores[1]			Number of judgments		
	Unirrad.	Irrad.	Signif. level	Correct	Incorrect	Signif. level
Sponge cakes						
1	5.27	5.36	(2)	10	10	Not
2	5.92	4.77	(2)	11	9	5%
3	6.23	2.38	(2)	13	7	1%
4	5.92	5.14	Not	10	10	Not
5	5.71	3.59	0.1%	14	6	0.1%
6	5.50	3.50	5%	13	7	1%
Custards						
1	5.63	2.94	(2)	16	4	0.01%
2	6.25	3.00	(2)	12	8	5%
3	5.07	3.71	Not	14	6	0.1%
4	5.93	2.65	0.1%	14	6	0.1%
5	6.71	3.39	0.1%	18	2	0.01%
Scrambled eggs						
1	4.62	2.75	(2)	14	6	0.1%
2	4.55	2.25	0.1%	20	0	0.001%

[1] The taste panel rated the samples for acceptability according to the following scores: Score #1, dislike extremely, Score #2, dislike very much, Score #3, dislike moderately, Score #4, dislike slightly, Score #5, neither like nor dislike, Score #6, like slightly, Score # 7, like moderately, Score #8, like very much, Score #9, like extremely.

[2] (2) — Not calculated.

TABLE 5

Acceptability scores for sponge cakes and custards made with irradiated (850,000 rep) and unirradiated commercially dried egg yolk solids

Sample	Acceptability scores[1]			Number of judgments		
	Unirrad.	Irrad.	Signif. level	Correct	Incorrect	Signif. level
Sponge cakes						
1	5.84	4.46	(2)	12	8	5%
2	4.12	3.44	(2)	10	10	Not
3	5.14	4.28	Not	13	5	0.1%
4	6.06	4.82	5%	14	7	1%
5	5.75	4.58	Not	9	11	Not
6	4.82	3.28	Not	10	10	Not
Custards						
1	5.41	2.75	(2)	16	4	0.01%
2	5.00	3.09	(2)	11	9	5%
3	4.20	2.60	5%	13	6	0.1%
4	6.05	3.95	0.1%	19	2	0.01%
5	6.06	2.75	0.1%	16	4	0.01%

[1] See Table 4 for explanation of scores.
[2] (2) — Not calculated.

ever, the time required to reach a maximum consistency was greater (14 minutes) with irradiated solids than with unirradiated solids (8 minutes). In addition, the maximum consistency of the irradiated product was at a

TABLE 6

Acceptability scores for sponge cakes and custards made with irradiated (600,000 rep) and unirradiated commercially frozen egg yolk

Sample	Acceptability scores[1]			Number of judgments		
	Unirrad.	Irrad.	Signif. level	Correct	Incorrect	Signif. level
Sponge cakes						
1	5.36	5.91	(2)	9	11	Not
2	5.62	5.75	(2)	8	12	Not
3	5.93	5.73	Not	11	9	5%
4	6.67	5.08	5%	8	12	Not
5	6.44	5.12	5%	13	7	1%
Custards						
1	7.11	3.00	(2)	18	2	0.01%
2	6.38	3.75	(2)	7	13	Not
3	6.13	3.80	1%	13	7	1%
4	7.00	3.79	0.1%	12	8	5%
5	6.94	4.12	0.1%	15	5	0.1%

[1] See Table 4 for explanation of scores.
[2] (2) — Not calculated.

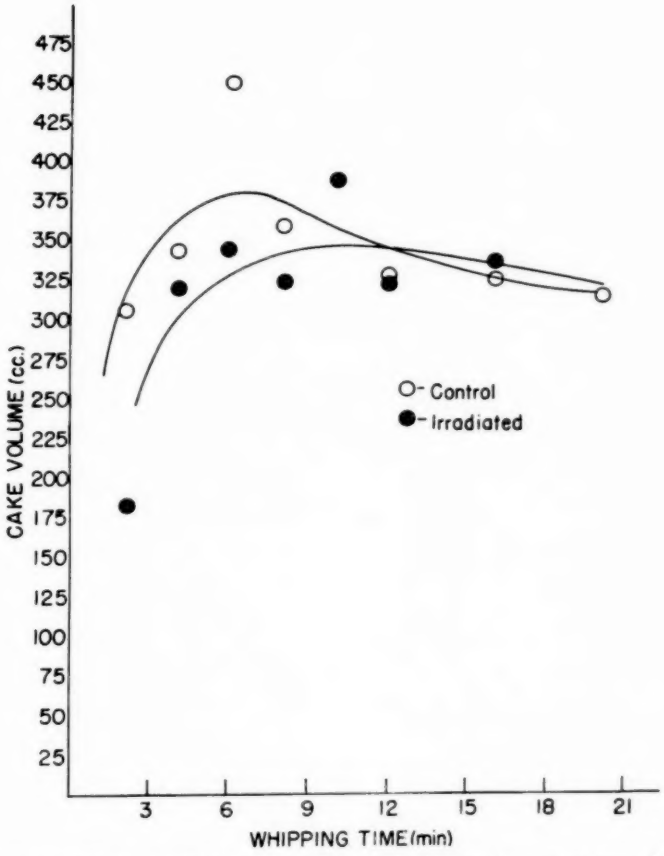

Figure 1. Effects of cathode ray irradiation and whipping time on volume of sponge cakes made with egg yolk solids.

TABLE 7

Effect of high-voltage cathode rays (850,000 rep) on volume of sponge cakes made with frozen egg yolk

Whipping time	Cake volume	
	Unirrad.	Irrad.
(min.)	*(cc.)*	*(cc.)*
1	319	329
2	362	369
4	415	409
8	369	395
12	297	299
16	243	254

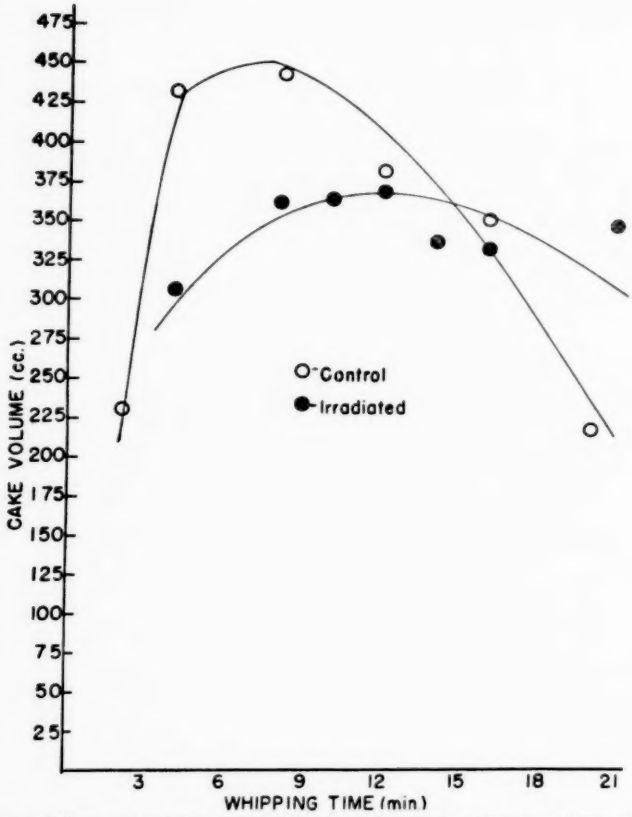

Figure 2. Effects of cathode ray irradiation and whipping time on volume of sponge cakes made with whole egg solids.

higher level than the maximum consistency of unirradiated product. The specific gravity of unirradiated material was at a minimum after 7 minutes whipping and of irradiated material after 10 minutes whipping.

The consistency of irradiated (850,000 rep) whole egg solids after reconstitution and whipping (Figure 4) was slightly lower than that of the unirradiated material at whipping times from 3 to 14 minutes. However, both materials increased in consistency at the same rate until the 14 minute point was reached. After 14 minutes whipping time, the rate of increase in consistency of the unirradiated reconstituted whole egg solids decreased slowly, reaching a constant value after 20 minutes whipping. In contrast, the consistency of the irradiated whole egg solids continued to increase at a high rate even after 24 minutes whipping. The specific gravity of irradiated whole egg solids decreased to a minimum after 8 minutes of whipping, whereas the minimum specific gravity of unirradiated material was achieved after 6 minutes whipping time.

TABLE 8

Effect of high-voltage cathode rays (850,000 rep) on whipping characteristics of commercially frozen egg yolk

Whipping time	Specific gravity		Reciprocal of distance flowed in 60 sec.	
	Unirrad.	Irrad.	Unirrad.	Irrad.
	(gm./cc.)	*(gm./cc.)*	*(cm^{-1})*	*(cm^{-1})*
2	0.632	0.530	0.101	0.107
3	0.454	0.387	0.080	0.116
4	0.363	0.371	0.105	0.153
5	0.326	0.352	0.147	0.168
6	0.285	0.334	0.206	0.200
7	0.305	0.241	0.241	0.260
8	0.315	0.359	0.225	0.215
9	0.331	.....	0.234	.....
10	0.346	0.430	0.198	0.211
12	0.392	0.314	0.212	0.200
14	0.455	.....	0.228	.....
15	0.451	0.462	0.191	0.213
16	0.478	.....	0.217	.....
17	.....	0.516	.....	0.222
18	0.650	.....	0.253	.....
20	0.583	.....	0.267	.....

Results of peroxide tests made on ether-extractable oil from unirradiated and irradiated (850,000 rep) whole egg solids, egg yolk solids, and frozen egg yolk are given in Table 9. All peroxide values were small even though there appeared to be significantly more peroxides in irradiated than in unirradiated samples.

Fluorescence determinations made on whole egg solids showed significant increases in fluorescence as a result of irradiation at 850,000 rep (Table 9).

Effect of ascorbyl palmitate addition to frozen egg yolk prior to irradiation. Ascorbyl palmitate was added to thawed frozen egg yolk at concentrations of 0.5% prior to irradiation at 1,000,000 rep. Custards were prepared from this material and compared organoleptically with custards prepared from unirradiated frozen egg yolk and frozen egg yolk irradiated at 1,000,000 rep without the addition of ascorbyl palmitate. The results (Table 10) of these organoleptic comparisons vary considerably and are therefore

TABLE 9

Effect of high-voltage cathode rays (850,000 rep) on fluorescence and peroxide number of egg products

Type of egg	Fluorescence		Peroxide value	
	Unirrad.	Irrad.	Unirrad.	Irrad.
	(%T)	*(%T)*	*(mM/gm.)*	*(mM/gm.)*
Whole egg solids	13.7	21.2	3.85	5.70
Egg yolk solids	.....	.....	3.86	5.06
Frozen egg yolk	.....	.....	1.25	1.76

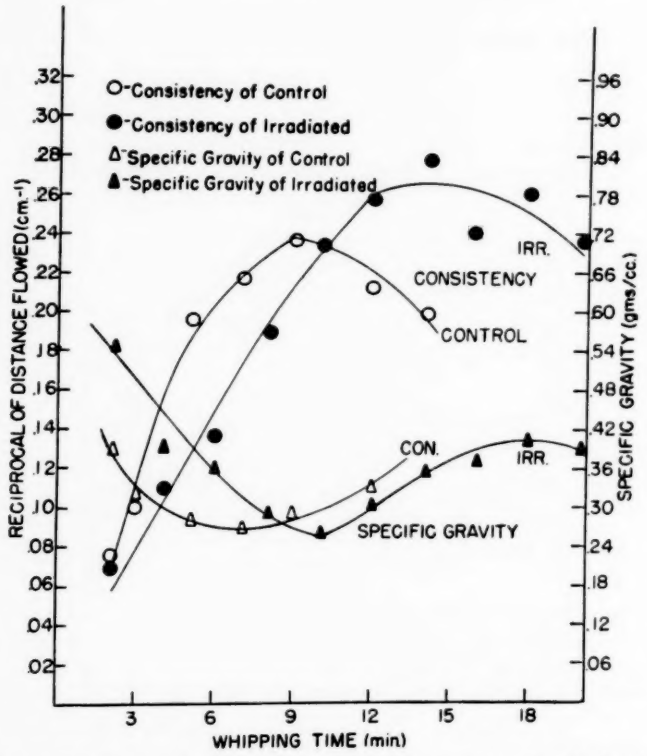

Figure 3. Effects of cathode ray irradiation (850,000 rep) and whipping time on consistency and specific gravity of egg yolk solids.

difficult to interpret. However, in general, it would appear that the addition of ascorbyl palmitate to this product was somewhat effective in lessening the irradiation off-flavor. One organoleptic comparison was made between two sets of custards prepared from unirradiated frozen egg yolk. One set of these custards contained 0.5% ascorbyl palmitate; the other did not. In this case, the custard containing palmitate was rated at a significantly lower score than the custard without palmitate addition. This indicates that palmitate itself adds an undesirable flavor to custard and enhances the observation that palmitate has a desirable effect toward the elimination of off-flavor due to irradiation.

Effect of lyophilization and spray drying after irradiation. Lyophilization of whole egg and egg yolk after irradiation was partially effective in alleviating irradiation off-flavor, when tested with both sponge cakes and custards (Tables 11 and 12). The spray drying of irradiated whole egg practically eliminated off-flavor due to irradiation even when custards were used as the test material. Only one test was carried out on irradiated, spray dried egg yolk, but this also indicated that this procedure was very effective in eliminating irradiation off-flavor.

TABLE 10

Effect of addition of ascorbyl palmitate on acceptability scores of custards made with irradiated (1,000,000 rep) and unirradiated frozen egg yolk

Test	Control sample	Test sample	Acceptability scores[1]			Number of judgments		
			Control	Test	Signif. level	Correct	Incorrect	Signif. level
1	Unirrad.	Irrad. +0.5% palmitate	5.09	3.73	5%	10	10	Not
	Irrad.	Irrad. +0.5% palmitate	4.44	4.33	Not	7	13	Not
2	Unirrad.	Irrad.	6.25	5.05	Not	(2)	(2)	(2)
	Unirrad.	Irrad. +0.5% palmitate	6.25	6.10	Not	(2)	(2)	(2)
3	Unirrad.	Irrad.	6.30	3.80	0.1%	(2)	(2)	(2)
	Unirrad.	Irrad. +0.5% palmitate	6.30	4.30	0.1%	(2)	(2)	(2)
4	Irrad.	Irrad. +0.5% palmitate	3.75	5.06	5%	15	5	0.1%
5	Irrad.	Irrad. +0.5% palmitate	4.36	4.79	Not	11	9	5%
6	Unirrad.	Irrad. +0.5% palmitate	5.65	4.17	5%	16	4	0.1%
7	Unirrad.	Irrad. +0.5% palmitate	5.73	4.87	Not	13	7	1%
8	Unirrad.	Irrad.	6.35	3.05	0.1%	(2)	(2)	(2)
	Unirrad.	Irrad. +0.5% palmitate	6.35	5.25	Not	(2)	(2)	(2)
	Irrad.	Irrad. +0.5% palmitate	3.05	5.25	0.1%	(2)	(2)	(2)
9	Unirrad.	Irrad.	6.45	4.75	5%	(2)	(2)	(2)
	Unirrad.	Irrad. +0.5% palmitate	6.45	5.05	5%	(2)	(2)	(2)
10	Unirrad.	Irrad.	6.65	4.35	0.1%	(2)	(2)	(2)
	Unirrad.	Irrad. +0.5% palmitate	6.65	4.95	1%	(2)	(2)	(2)
11	Unirrad.	Unirrad. +0.5% palmitate	5.64	4.00	1%	13	7	1%

[1] See Table 4 for explanation of scores.
[2] (2) — Not run as triangle test.

SUMMARY AND CONCLUSIONS

There was very little difference in the resistance of *Salmonella* to irradiation in whole egg solids, egg yolk solids, and frozen egg yolk. The average dose of high-voltage cathode rays (3 m.e.v.) required to cause a 10^7-fold destruction of *S. typhimurium* and *S. senftenberg* in whole egg solids was found to be about 450,000 rep, and in egg yolk solids about 650,000 rep. With frozen egg yolk, the effective dose for *S. typhimurium* and *S. senftenberg* was from 360,000 to 400,000 rep.

Atmospheres of air, oxygen and nitrogen had little or no effect on the resistance of these organisms to irradiation. It would appear to require only about a 50% increase in the 10^7-fold reduction dose to obtain 10^{11}-fold

TABLE 11

Effects of spray drying and lyophilization on acceptability scores of sponge cakes and custards made with irradiated (850,000 rep) and unirradiated whole egg

Treatment	Acceptability scores[1]			Number of judgments		
	Unirrad.	Irrad.	Signif. level	Correct	Incorrect	Signif. level
Sponge cakes						
Spray drying	6.10	5.00	Not	10	10	Not
Spray drying	4.84	4.16	Not	9	11	Not
Spray drying	5.86	4.00	1%	13	7	1%
Spray drying	5.60	5.00	Not	9	11	Not
Spray drying	6.13	5.38	Not	7	13	Not
Lyophilization	4.85	4.92	Not	12	7	5%
Lyophilization	6.00	4.69	Not	9	11	Not
Lyophilization	6.30	6.30	Not	5	15	Not
Lyophilization	6.67	4.56	1%	7	13	Not
Lyophilization	4.26	6.20	5%	14	6	0.1%
Lyophilization	6.21	5.21	5%	12	8	5%
Custards						
Spray drying	5.10	4.20	Not	9	11	Not
Spray drying	4.30	3.70	Not	8	12	Not
Spray drying	4.73	3.91	Not	9	11	Not
Lyophilization	4.72	3.88	Not	8	12	Not
Lyophilization	6.23	4.84	Not	11	9	5%
Lyophilization	5.93	3.63	0.1%	14	6	0.1%
Lyophilization	5.40	2.40	0.1%	14	6	0.1%
Lyophilization	6.14	4.73	5%	12	8	5%
Lyophilization	6.44	4.84	1%	10	9	Not

[1] See Table 4 for explanation of scores.

TABLE 12

Effects of spray drying and lyophilization on acceptability scores of sponge cakes and custards made with irradiated (850,000 rep) and unirradiated egg yolk

Treatment	Acceptability scores[1]			Number of judgments		
	Unirrad.	Irrad.	Signif. level	Correct	Incorrect	Signif. level
Sponge cakes						
Spray drying	4.71	5.00	Not	6	14	Not
Lyophilization	4.85	4.23	Not	12	8	5%
Lyophilization	4.00	3.55	Not	9	11	Not
Lyophilization	5.75	5.16	Not	11	9	5%
Lyophilization	6.21	4.28	1%	12	8	5%
Lyophilization	5.62	4.88	Not	18	2	0.01%
Custards						
Spray drying	4.00	3.75	Not	11	9	5%
Lyophilization	5.18	3.73	5%	11	9	5%
Lyophilization	4.55	3.77	Not	8	12	Not
Lyophilization	5.76	2.59	0.1%	17	3	0.1%
Lyophilization	7.14	4.53	0.1%	13	7	1%

[1] See Table 4 for explanation of scores.

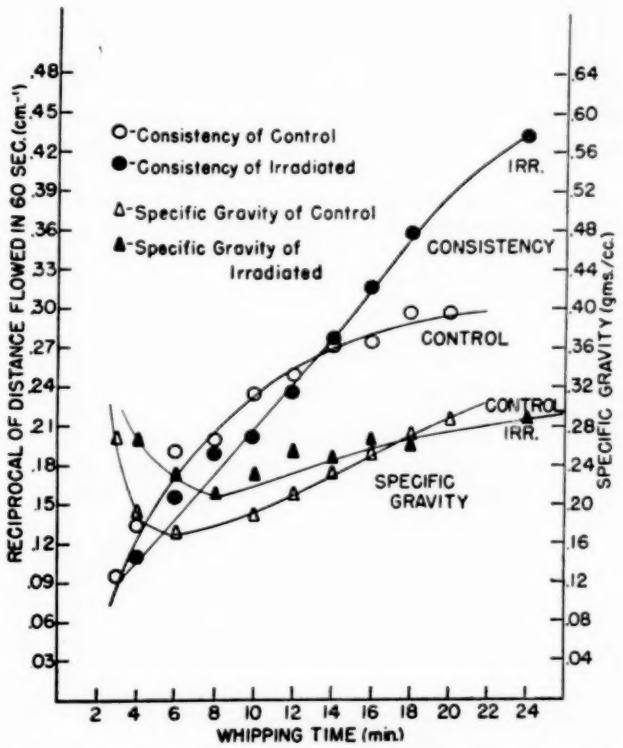
O-Consistency of Control
●-Consistency of Irradiated
Δ-Specific Gravity of Control
▲-Specific Gravity of Irradiated
IRR.
CONSISTENCY
CONTROL
CONTROL
IRR.
SPECIFIC GRAVITY
RECIPROCAL OF DISTANCE FLOWED IN 60 SEC.(cm.⁻¹)
SPECIFIC GRAVITY (gms./cc.)
WHIPPING TIME (min.)
.48 .45 .42 .39 .36 .33 .30 .27 .24 .21 .18 .15 .12 .09 .06 .03
.64 .60 .56 .52 .48 .44 .40 .36 .32 .28 .24 .20 .16 .12 .08 .04
2 4 6 8 10 12 14 16 18 20 22 24

Figure 4. Effects of cathode ray irradiation and whipping time on consistency and specific gravity of whole egg solids.

reduction, which may be considered a reduction factor comparable to that used in the canned food industry.

When unflavored sponge cakes were used to evaluate the flavor changes due to irradiation of frozen egg yolk, whole egg solids and egg yolk solids, a significant, but minor, effect of irradiation was noted. When unflavored custards were used as the test material the flavor differences were highly significant.

For both egg yolk solids and whole egg solids, the volumes of sponge cakes prepared from irradiated material were somewhat lower than the volumes of cakes prepared from unirradiated materials. An increased whipping time was required to reach maximum volume when irradiated egg solids were used, as compared with unirradiated solids. In the case of frozen egg yolk there was little difference in volumes of sponge cakes prepared from irradiated and unirradiated materials.

Irradiation of frozen egg yolk had little, or no, effect on either the consistency, or specific gravity of whipped material. The relationship between consistency and performance of egg products of this kind is not known.

Determinations were made of the effect of irradiation on fluorescence and peroxide number. There was significantly more peroxide in the oil of all irradiated yolk or whole egg samples tested than in the unirradiated products. The fluorescence of whole egg solids increased slightly upon irradiation.

Addition of ascorbyl palmitate to frozen egg yolk before irradiation had a significant, but minor, effect in lessening the production of radiation off-flavors.

Spray drying of egg yolk and whole egg after irradiation in the liquid state was very effective in lessening radiation off-flavor, and lyophilization of these products after irradiation was partially effective. It would thus appear to be technically feasible to irradiate the egg material in the liquid state and then to spray dry. By this method, the irradiation off-flavor would be largely obviated.

An irradiation dose causing a 10^7-fold reduction in *Salmonella* organisms in frozen egg yolk, whole egg solids, and egg yolk solids was found to cause only minor deteriorations in the functional properties of these products. Flavor changes were found to be minor when sponge cakes were used as the test material, but highly significant when custards were used to evaluate flavor. Spray drying of egg yolk and whole egg after irradiation largely offset the off-flavor caused by irradiation.

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NOTES ON SOME SOUTHERN RHODESIAN INDIGENOUS FRUITS, WITH PARTICULAR REFERENCE TO THEIR ASCORBIC ACID CONTENT

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The edible indigenous fruits of Southern Africa have been largely neglected by both nutritionist and horticulturist, and no attempt has been made to investigate their suitability or value for development or cultivation. As a result, Southern African fruit industries rely almost entirely on varieties which have been imported from many parts of the world.

In the early days of this century, the Imperial Institute investigated a number of different fruits found in Southern Africa, and in the Union of South Africa, Marloth *(7)* carried out analyses on many of the fruits common to both territories. However, the experimental methods used then were obviously limited in scope, and little is known of the nutritional value of these fruits.

As a result of the discovery of relatively high concentrations of ascorbic acid in the pith of the baobab fruit, *Adansonia digitata* L., *(4)*, it was decided to carry out a limited survey on as many as possible of the fruits known to be edible and growing wild in the veld of Southern Rhodesia, in the hope that it may prove possible to propagate and develop suitable varieties.

At this stage, some notes on the geography and climate of the country are necessary. Southern Rhodesia, the southernmost part of the Federation of Rhodesia and Nyasaland, lies entirely in the Southern tropics of Africa. It is bounded in the North by the Zambesi River, in the south by the Limpopo River, and in the east and west by Moçambique and Bechuanaland Protectorate respectively. Much of the Colony is a plateau, the altitude varying between 900 m. to 1,500 m. above sea level with a mean annual rainfall of 63 cm. (25 in.) to 100 cm. (40 in.) most of which falls in the summer months of November to April.

The climate of the plateau can be regarded as subtropical, with occasional heavy frosts at night in the winter months. The remainder of the Colony (the low veld) is very much hotter and has a lower rainfall.

COLLECTION OF SAMPLES

Samples were collected from all over the Colony, with particular emphasis on the low veld areas. Co-operation was secured from members of the public and Government officials, who were supplied with sampling instructions and polythene bags for the storage of the fruits.

Samples were transmitted to the laboratory by the quickest available means and stored in a refrigerator for a maximum of 24 hours before analysis. It proved impossible to obtain more than one sample of many of the fruits, mainly due to the small number which were ripe at any one time, the widely scattered nature of the trees or shrubs, and the popularity of the fruits with the younger indigenous inhabitants.

Botanical identification was carried out by the Federal Ministry of Agriculture Department of Botany and with the aid of Wild's Botanical Dictionary *(9)*.

DESCRIPTION OF FRUITS AND THEIR USES

All the varieties collected are well known and popular with Africans, especially the children, although none are used commercially.

1. *Parinari mobola* Planch. (cork-tree, Hissing tree) is a common, medium sized to tall evergreen tree bearing ovoid, dull brown fruit with a roughish skin about 3 cm. long. The fruit may be eaten fresh or pulped with water to make into a drink.

2. *Azanza garckeana* (F. Hoffm.) Excell and Hillcoat (quarters, snot-apple) is a widely distributed small tree with a globose, woody fruit capsule up to 4 cm. in diameter with a bluntly pointed apex. The capsule is divided into 4 sections and has an edible pulp around the seeds containing a mucilage. The pulp has a pleasant, sweet flavour when chewed and is particularly popular with children.

3. *Ficus capensis* Thunb. (cape fig, wild fig) is a common tree with a milky latex, growing up to 15 m. in height. The figs are borne on woody, panicled, leafless branches on the main stem and are obvoid or obvoid-globose and glabrous with a prominent ostiole at the apex. They are pinkish-green and fleshy when ripe and commonly infested with insects and ants. They are not a very popular fruit, mainly due to their usual heavy infestation. When eaten, the many small pips are usually rejected.

4. *Strychnos* spp. (Kaffir orange, monkey orange) are widespread in Southern Rhodesia. The following species were investigated:

S. spinosa Lam. is a small tree with smoothish dark bark. The fruit is yellow when ripe and the size of a large orange. The skin is hard and has to be cracked open, whereupon the pulp, with tough white seeds embedded, may be removed as a whole. The pulp is dark brown in colour, with an acid flavour. The seeds are rejected when eaten. The juice is sometimes incorporated in a porridge made from maize.

S. cocculoides Bak. is similar to *S. spinosa* with a very corky bark. It is sweeter than the latter and consequently more popular.

S. innocua Del., the most popular of the *Strychnos,* has a smaller fruit with a softer shell. It is much sweeter than the other two and has a pleasanter flavour.

5. *Uapaca kirkiana* Muell. Arg. (wild loquat) is a widespread and common small tree, often growing in groves. The fruit is a sub-globose, brown fleshy berry the size of a plum. The flesh has a sweet, pleasant flavour and has large seeds embedded in it. The skin, and pulp associated with it, are very astringent and require removal before eating. This is a very popular fruit over wide sections of the eastern half of the high veld, and is one of the few indigenous fruit offered for sale at village stores.

6. *Lannea edulis* Engl. (wid grape) is a widely spread common perennial, sending up leaves annually from a large woody rootstock. The fruit changes from red to blue-black when ripe, and has a large seed in the centre. It is very popular with children, but difficult to obtain in any quantity due to the small numbers ripening at any one time.

7. *Lannea discolor* (Sond.) Engl. (live long) is a widely distributed

common small tree up to 7 m. in height. It is seen in the low veld and lower parts of the high veld. The fruit is a small ovoid reddish drupe about 1 cm. long. It is very popular, but liable to attack by birds. There is little flesh on the fruit, as it contains a large seed which is rejected with the skin.

8. *Ximenia caffra* Sond. (sour plum, monkey plum) is a common widespread shrub or small tree, often found on termite mounds. It is common to both high and low veld areas. The fruit is an oblong drupe, bright red when ripe and the size of a small plum. It is very sour, with a slight "bitter almond" flavour, and is not very popular.

9. *Tamarindus indica* L. (tamarind) is a tall tree up to 20 m. in height. It is found only in the Zambesi valley area near rivers. The fruit is sausage shaped and about 12 cm. long with a ferruginous or greyish powdery skin. The pulp surrounding the seed is edible and is sticky and sweet flavoured. The outer skin is removed and the sticky pulp containing the seeds placed in water or milk, sometimes with added wood ash. The whole is stirred and then the seed allowed to settle. The supernatant liquid is poured off and used as a drink. It is a popular substitute for sugar or honey.

10. *Cordyla africana* Lour. (wild mango) is a very large, handsome tree common in the Sabi and Zambesi valleys. The fruit is fleshy, rather like a ripe mango in shape and colour, with about 2 large bean-like seeds embedded in the flesh. Both pulp and seeds are eaten, the seeds after drying over a fire. Only the pulp has been considered in this survey.

11. *Vangueriopsis lanciflora* (Hiern) Robyns is a widely distributed shrub or small tree mostly in low veld areas. It flowers before the leaves appear and the young branches are thick and orange-red in colour. The fruit is dark brown in colour, and shaped rather like a fig. It is eaten fresh and is wholly edible.

12. *Garcinia buchananii* Bak, is a small to moderate sized tree, not common, but widely distributed on granite hills. The yellow, plum-like fruit has juicy yellow flesh with 4 bean-like seeds. It is eaten fresh and the skin and seeds discarded.

13. *Syzygium guineense* DC (water boom, water berries) is a medium sized tree, common along river banks. The fruit is oval, fleshy and purple in colour. The purple skin is easily peeled, exposing a white flesh with a large seed in the centre. The trees very often bear a heavy crop which is particularly popular with children.

14. *Vitex payos* (Lour.) Merill is a common and widespread small tree. The fruit is black skinned with brown, rather dry flesh enclosing a large seed. It is about 3 cm. long, ovoid and oblong. The flesh has a very distinctive sweet flavour and is very popular with Africans.

15. *Scherocarva birrea* Sond. (Marula plum) is a tall tree common in the low veld and the western border of the Colony. The fruit is a drupe about 8 cm. in diameter with a pale green skin. It ripens on the ground to a fawn colour and eventually ferments. The flesh is white and very juicy, rather similar to a Lychee, with a large seed in the centre. The trees very often bear a heavy crop and are very distinctive in the fruiting season, due to the large numbers of fruit lying round the trunk. The fruits find wide use in the manufacture of an alcoholic drink in the villages, besides being eaten fresh. A common practice in many areas is to incorporate the juice into a thin

porridge with maize meal, and there are some Afrikaner recipes for the preparation of very pleasant jams and jellies. These recipes are not well known, however, over much of the Colony. Elephants and bush pigs regard the fruit as a delicacy, and rumour has it that these animals have been known to become quite drunk after eating fermented marulas. In many villages, the seeds are collected, sundried and the shell cracked open. The nuts are eaten fresh or roasted or ground into a paste. Similar preparations are known in other territories, particularly Nyasaland as Williamson *(10)* shows in her book. She also mentions the high vitamin C content of the beer prepared from the marula which is confirmed in the results obtained in this survey.

16. *Flacourtia indica* (Burm. F) Merr. (Batoka plum, Rhodesian plum) is a common shrub or small tree with rough greyish-brown bark, often with sharp spines. The fruit is about 3 cm. in diameter with a purple red skin. The pulp is fleshy and contains about six small seeds, which are rejected when eaten, together with the skin. This is quite a popular fruit and is usually eaten straight from the tree, which very often bears a heavy crop.

17. *Vangueria tomentos* Hochst. (small wild medlar) is a common and widespread shrub or small tree, very similar to *Vangueriopsis lanciflora*, except that the fruit is smaller.

18. *Ampelocissus asarifolia* Planch. (wild grape) is a widespread climber particularly in the low veld. The fruit is red coloured and rather cherry-like, about 1.5 cm. in diameter. It is formed in bunches on long stems. Although widespread, this climber is not very common, and many Africans are not aware that the fruit is edible.

19. *Phyllogeiton discolor* (Klotzch.) Herzog is a moderately sized tree growing up to 10 m. quite common in the low veld, particularly the Zambesi Valley. The fruit is a small yellow ovoid drupe about 1 cm. in diameter, with a large seed in the centre, which is rejected. The fleshy part is moist with a pleasant sweet flavour, and is sometimes used for making an alcoholic drink.

20. *Royena sericea* Bernh. (monkey plum) is a common, much branched shrub or small tree, often found on termite mounds. The fruit is ovoid, reddish or yellow when ripe and some 2 cm. in diameter. The skin is easily peeled, showing colourless transparent flesh with one to three black seeds. This fruit is regarded with some suspicion by certain Africans, apparently on superstitious grounds.

Many of these fruits are well known over much of Africa, south of the Sahara, and reference to their edibility and methods of food preparation in other parts of Africa can be found in works by Dalziel *(5)*, Eggeling *(6)* and Williamson *(10)*.

EXPERIMENTAL PROCEDURE

The fruit was weighed before analysis and the edible portion of each fruit was determined in a variety of ways, depending on individual peculiarities, and can only be regarded as a guide.

The remaining figures were determined as far as possible on the edible portion, but in some cases the fruit was too small, or in too short supply, to make it possible to separate the edible portion and the analysis was carried out on the whole fruit.

Moisture determination was carried out by heating for 4 hours at 100° C. or, for whole fruit, by the Dean and Stark method of distillation with an immiscible liquid.

Sugars were determined before and after inversion by the Fehlings volumetric method of Lane and Eynon as given in the A.O.A.C. *Methods of Analysis (1)* and were expressed

as invert sugar and sucrose. No attempt was made to investigate the nature of the carbohydrates present.

Ascorbic acid was determined by the 2,6-dichlorophenolindophenol visual titration method, as originally described by Bessey and King *(3)* in all the samples. Those samples showing promisingly high values were assayed by the 2,4-dinitrophenylhydrazine method of Roe and Oesterling *(8)* for reduced ascorbic acid, dehydroascorbic acid and diketogulonic acid. The technique described by the Association of Vitamin Chemists *(2)* was followed in both cases.

RESULTS AND DISCUSSION

The results obtained are expressed in Tables 1 and 2, and it can be seen that many of the fruits are small, with a large inedible seed, giving a very small edible portion. This, combined with their comparative rarity, and small cropping, makes them of insignificant nutritional value. Other fruits, such as the wild loquat and Kaffir orange, are popular and have a pleasant flavour and heavy yield but are very low in ascorbic acid.

Of the fruit containing appreciable quantities of ascorbic acid, the sour plum (*Ximenia caffra.* Sond) is so sour that it is almost impossible to eat more than one, the wild mango (*Cordyla africana* Lour.) is confined to a comparatively small area of the Colony and is a fruit of doubtful popularity, and *Phyllogeiton discolor* (Klotzch.) Herzog, whilst very popular, is a comparatively rare fruit, and it proved impossible to obtain more than one sample.

The remaining two fruits of high ascorbic acid content are in a different category; both are widespread and both are popular. *Parinari mobola* Oliv. is commonly left standing when a new area is opened for cultivation, as it is also a useful shade tree, and it is therefore to be found in areas where much of the indigenous timber has been destroyed and the Marula (*Sclerocarya birrea.* Sond) is also treated in this manner. The ascorbic acid content in both cases shows considerable variation, and is particularly complicated in the case of the Marula by variations in the amount of reduced ascorbic acid present, as is seen in Table 2.

The Marula, however, shows very interesting properties. The total ascorbic acid remains remarkably stable at all stages. The fruit falls from the tree whilst still green, ripens on the ground and eventually ferments, and through all these stages, the ascorbic acid remains largely constant, and when the pulp was removed and stored on the bench with no precautions, Table 2 shows that there was no loss of the vitamin although the pulp fermented violently and eventually became acid and mouldy over the 22 days.

This stability is reflected in the figures obtained for beer and jelly prepared from the fruit, which indicate reasonably accurately the amount of fruit in the preparations.

The Marula takes about 15 years to bear fruit and investigations are proposed to determine the possibility of cultivating this tree to produce heavy crops in a shorter period and of high ascorbic acid and sugar content.

SUMMARY

A description of a number of indigenous fruit trees and shrubs of Southern Rhodesia is given and the nature of the fruits indicated. The ascorbic acid content, weight, edible portion, moisture and sugar content of the fruit is

TABLE 1

Fruit data with reduced ascorbic acid figures

Botanical Name	Common Name[1]	Average Weight g.	Edible Portion %	No. of Samples	Analysis Sample	Mean Moisture %	Mean Sugars as		Indophenol Method Ascorbic Acid Content		
							Invert Sugar %	Sucrose %	Average mg/100g	Minimum mg/100g	Maximum mg/100g
Ampelocissus asarifolia	Wild grape umHlambi (Ndebele)	2.2	.....	1	Whole fruit	83.9	3.3	0	trace	.....	.....
Azanza garckeana	quarters, snot apple muTohwe (Shona)	44	85 'chewable'	1	Edible portion, ripe ("chewable")	18.0	36.8	2.6	0	.....	.....
Cordyla africana	Wild mango muTondo (Ndau)	14.4	45	1	Pulp and seeds (early windfalls)	80.9	trace	0	75.6	.....	.....
Ficus capensis	Cape fig muKuyu (Shona)	27	100	3	Whole fruit	85.0	6.3	0	trace	.....	.....
Flacourtia indica	Batoka plum umQuokolo (Ndebele)	6.1	61	3	Edible portion, ripe	72.3	13.7	0	0	.....	.....
Garcinia buchananii	muKute (Shona)	11.1	54	1	Edible portion, ripe	75.9	.....	....	8.0	.....	.....
Lannea discolor	live long muPuri (Ndau)	0.3	32	1	Whole fruit	51.9	.....	....	5.0	.....	.....
Lannea edulis	wild grape muTsambatsi (Shona)	1.0	32	2	Whole fruit	70.5	4.7	0	14.0	10.0	18.1
Parinari mobola	Hissing tree muHacha (Shona)	21	50.5	3	Edible portion, ripe	69.1	5.7	7.2	64.1	17.6	126.2
Phyllogeiton discolor	Mu We (Tonga)	.....	....	.....	Whole fruit	72.5	18.8	0	87.0	.....	.....
		0.9	74	1	Edible portion, ripe	.....	.....	....	116.0	.....	.....
Royena sericea	Monkey plum umCatuya (Ndebele)	1.3	39	1	Whole fruit	63.5	7.7	0.4	32.0[2]	.....	.....
Sclerocarya birrea	Marula plum muRula (Shona)	31	30	7	Edible portion, ripe	87.0	2.3	5.9	179.1	62.3	254.0
		34	....	1	E.P., ripe } from same tree	86.1	1.9	6.8	133.7	.....	.....
		39	....	1	E.P., unripe } from same tree	87.2	1.2	6.5	127.2	.....	.....
		.....	....	1	E.P., overripe } from same tree	87.4	2.3	2.5	52.7	.....	.....
		.....	....	2	Beer	.....	.....	....	97.1	89.7	104.5
		.....	....	4	Jelly	.....	31.2	39.2	69.4	65.2	76.2
		.....	....	1	Jam	.....	31.4	35.1	105.0	.....	.....
Strychnos spinosa	Kaffir orange muTamba (Shona)	424	50	2	Edible portion, ripe	82.1	11.0	0	1.45	1.4	1.5
S. cocculoides	Kaffir orange muTamba (Shona)	219	42	2	Edible portion, ripe	79.7	9.7	4.8	1.2	1.0	1.4
S. innocua	Kaffir orange muHwakwa (Shona)	69	40.5	1	Edible portion, ripe	70.6	7.2	7.4	14.4	.....	.....
Syzygium guineense	Water berry muKute-rusumbudzi (Shona)	1.6	50	1	Edible portion, ripe	88.1	8.4	0	0	.....	.....
Tamarindus indica	Tamarind muSeka (Shona)	16.6	64	1	Pulp and seeds	20.7	19.4	0.4	0	.....	.....
Uapaca kirkiana	Wilk loquat maHobohobo (Ndebele)	22	38.5	4	Edible portion, ripe	76.0	5.8	2.7	1.8	0	5.2
Vangueria tomentosa	small wild medlar umViyo (Ndebele)	3.2	100	1	Whole fruit	53.1	10.2	0	0	.....	.....
Vangueriopsis lanciflora	umViyo (Ndebele)	17.7	100	1	Whole fruit	78.4	3.9	0	0	.....	.....
Vilex payos	muTsubvu (Shona) umChangwela (Ndebele)	1.5	47	2	Edible portion, ripe	50.5	6.2	0	0	.....	.....
Ximenia caffra	Sour plum muTswanzwa (Shona)	7.2	49	3	Whole fruit	66.4	trace	0	49.2	24.8	68.4

[1] The African dialect or language for the common names is given in parentheses.
[2] Total ascorbic acid.

TABLE 2

Comparison of reduced and total ascorbic acid. Ascorbic acid in mg./100 g. on the edible portion of the fruit pulp

Botanical Name	Analysis Sample	Ascorbic acid	
		Total	Reduced
Parinari mobola	freshly harvested	.....	126
	pulp refrigerated 4 days	123	36
Sclerocarya birrea	1a. freshly harvested	.....	206
	1b. fruit stored 7 days	265	114
	2a. freshly harvested	.....	201
	2b. fruit stored 14 days	316	294
	3a. freshly harvested	.....	62
	3b. fruit refrigerated 7 days	272	239
	4a. freshly harvested	275	254
	4b. pulp stored 24 hr.	.....	262
	48 hrs.	.....	266
	72 hrs.	.....	261
	22 days	.....	261
	5. jelly prepared from pulp	72	71

presented. *Sclerocarya birrea, Parinari mobola* and *Phyllogeiton discolor* were found to contain relatively large amounts of ascorbic acid.

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MEDIA FOR DETECTION OF PECTINOLYTIC MICROORGANISMS ASSOCIATED WITH SOFTENING OF CUCUMBERS, OLIVES AND OTHER PLANT TISSUES*

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In recent years our knowledge of the spoilage of cucumbers and olives commonly called softening has been materially increased. However, when it is considered that the microbial origin of such spoilage was suggested before 1900 *(1)*, progress has not been startling. There have been several deterrents. Knowledge of the complex chemistry of the pectinous materials, whose degradation is largely responsible for plant tissue softening, has been evolved slowly. Interest has been divided between these purely chemical aspects, the biological and biochemical interrelations of various microorganisms and their pectinolytic enzymes, and the isolation and characterization of intrinsic pectinolytic enzymes of higher plants including the cucumber. The greatest advances have been made comparatively recently *(4)*. Therefore, it is not surprising that the application of this newer knowledge to the softening of cucumbers and olives has been slow or that confusion still exists concerning the possible causes of their degradation. Furthermore, because of technical difficulties, actual proof of the etiology of a particular outbreak of softening frequently is lacking. The involved techniques generally used to demonstrate *in vitro* pectinolysis by microorganisms present the greatest obstacles because of the exacting, time-consuming requirements *(7)*.

A simple, direct screening medium for detection of pectinolytic organisms would obviate some of these difficulties. A polypectate gel medium offers promise in this respect. Baier and Manchester *(2)* introduced such a gel as a substitute for bacteriological agar which in 1943 was in short supply because of World War II. However, as a substitute for agar, use of the gel is restricted. Too many different microorganisms produce pectinolytic enzymes. Adjacent colonies of pectinolytic organisms fuse rapidly. One strongly pectinolytic colony may produce enough enzyme to cause liquefaction of a large sector of the gel in a petri dish, thus causing coalescence of all colonies in that area, irrespective of their individual pectinolytic properties. Therefore, if a sample contains a significant population of pectinolytic organisms, a quantitative plate count is impossible.

These observations suggested that the same *(2)* or a similar gel preparation *(4a, 6, 11, 17)* could be used for rapid detection of pectinolytic microorganisms. The original medium of Baier and Manchester *(2)* was made with sodium ammonium pectate. However, production had been changed to sodium polypectate by the time exploratory experiments had been completed by Vaughn, Kreulevitch, and Mercer *(15)* and Nortje and Vaughn *(14)*. Introduction

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of the sodium polypectate required reformulation of the medium. Vaughn, Levinson, Nagel, and Krumperman *(16)* reported that 60 g. of sodium polypectate were required to produce a good bacteriological gel in the neutral range (pH 6.5 to 7.0) whereas only 45 g. of sodium ammonium pectate per liter of medium had been sufficient before. During this latter investigation difficulties were experienced when studying the effect of the pH of the gel medium on growth of bacteria and molds. Further observations on preparation and use of polypectate media for rapid detection of pectinolytic microorganisms are described by the present authors.

EXPERIMENTAL METHODS AND RESULTS

Gel formation at different pH values. In the previous investigations *(14, 15, 16)* it had been very difficult to adjust the pH of the pectate gel medium to values well within the acid range (4.0 to 4.5). The same difficulty was experienced in this study. The adjustment of the complete medium either before or after autoclaving was not satisfactory because gelation occurred around each drop the moment acid (HCl) was added, regardless of its concentration.

Adjustments in the concentration of "Calgon" (sodium hexametaphosphate) had no beneficial effects on gelation at low pH values or near neutrality so it was concluded that enough of this compound already was present.

Since the pH value is of major importance for the proper gelation of pectin and pectate in the manufacture of jellies *(3, 7)* it was anticipated the gel formation would in this case be related to the pH value and the amount of sodium polypectate added to the medium. Experiments showed that gelation was a function of pH and sodium polypectate concentration. The lower the pH value, the less the amount of sodium polypectate required to produce a satisfactory bacteriological gel. This relationship was found to be almost linear when nutrient peptone beef extract was the basal medium. Therefore, the concentration of sodium polypectate required per 100 ml. of medium for good gelation is approximately equal to the pH value desired. At pH 4.5 this relationship is exact: 4.5 g. of sodium polypectate per 100 ml. are required to produce a satisfactory gel with the nutrient basal medium. However, with pH values near neutrality the amount of sodium polypectate required is approximately 10% less than that corresponding to the pH. In contrast for pH values between 3.5 and 4.0 the quantity of pectate has to be increased by 10%. These relationships are shown in Table 1.

Good gelation was obtained when the basal medium (5.0 g. Bactopeptone, 3.0 g. Bacto-beef extract, and 3.5 g. "Calgon", food grade per liter of water) was adjusted with 50% citric acid solution to a pH value 0.5 units lower than that desired in the sterile gel. After adjustment of the pH the mixture was heated to boiling and placed in a laboratory blender. The sodium polypectate (estimated on the basis of the desired pH according to Table 1) was added and blending was started. Mixing was continued until a homogenous slurry was formed. Then the medium was distributed in flasks or tubes and sterilized. At pH values between 4.5 and 7.0 the medium was heated at 15 p.s.i.g. for 15 minutes. When the pH values were from 4.0 to 4.5 the medium was sterilized at 10 p.s.i.g. for 10 minutes. The very acid medium (below pH 4.0) was heated in boiling water or streaming steam for 10 minutes in plugged containers previously autoclaved at 15 p.s.i.g. for 15 minutes. The pH value after heating always was within $\pm$ 0.1 unit of that expected.

Care must be taken in adjustment of the pH and determination of the amount of sodium polypectate required for gelation of the peptone, beef extract medium. An excess of the polypectate will cause gelation in the blendor whereas a deficit will result in poor gelation after sterilization, even when the pH is correctly adjusted.

The peptone, beef extract gel was quite satisfactory for demonstration of pectinolysis by various bacteria. The species tested included the aerobic species *Bacillus laterosporus, Bacillus pumilus, Bacillus subtilis,* and *B. subtilis* var. *aterrimus;* the facultative aerobic species *Bacillus macerans* and *Bacillus polymyxa;* the anaerobic species *Clostridium felsineum* and *Clostridium multifermentans;* and *Erwinia carotovora.* Strains of these species caused liquefaction of the gel at pH values at or near neutrality.

The medium also was useful for demonstrating the pectinolytic activity of a variety

TABLE 1

The initial pH and concentration of sodium polypectate required for satisfactory bacteriological gels with nutrient peptone beef extract, potato infusion plus glucose, and malt powder as basal media

Initial pH of basal medium [1]	Amount of sodium polypectate added to basal medium g./l.	pH of medium after sterilization [2]	Approximate change in pH, %
	Nutrient gel		
7.7	65–70	7.0	−10
7.2	60–63	6.5	−10
6.7	55	6.0	−10
6.1	52	5.5	−10
5.5	50	5.0	−10
4.5	45	4.5	no change
3.6	40	4.0	+10
3.2	38	3.5	+10
	Potato infusion, glucose gel		
6.5	64	5.9	−10
5.0	46	5.1	no change
4.5	45	5.0	+10
3.5	43	4.3	+20
2.5	34	3.3	+30
	Malt powder gel		
6.5	60	5.9	−10
5.0	45	5.0	no change
4.5	44	4.9	+10
3.5	40	4.2	+20
2.5	35	3.4	+35

[1] Adjusted with 50% aqueous citric acid.
[2] To the nearest tenth because the pH values always varied within ± 0.1.

of fungi as shown in Table 2. All of the fungi grew rapidly on this medium but the best development was observed at the higher pH values. The most abundant mycelium was formed by all cultures at pH values between 5.0 and 7.0. However, although the majority of the cultures grew at pH 3.3, the lowest point tested, 4 isolates of *Fusarium roseum*, one each of *Fusarium gibosum* and *Fusarium oxysporum* and the *Verticillium* and *Streptomyces* species failed to grow at pH values below 4.0 in this study.

The highest pH value at which softening was observed was 5.2. At a pH of 4.2 all of the isolates softened the medium except the unidentified culture of *Aspergillus* and *Paecilomyces varioti*. These latter cultures liquefied the gel only when the pH of the medium was 3.3. The optimum initial pH of the gel for softening by all isolates was between 3.3 and 4.2. It also was observed that in general the softening of the gel was the most rapid at the lowest pH. The unidentified *Oospora* isolate caused the most rapid liquefaction of the gel followed in turn by the cultures of *Penicillium* and *Fusarium moniliforme*, respectively, when they were grown at pH 3.3. However, as already noted, some cultures could not grow at pH values below 4.0.

It was also interesting to note that the pH of the medium after growth of the molds increased to 8.0 to 9.5 whether or not the gel was liquefied.

Gel formation with other basal media. Although the results with the peptone, beef extract gel just described indicate that this medium can be used for detection of pectinolysis by molds, other media have been used almost traditionally for the growth of molds and yeasts. Therefore, it was desirable to investigate the use of gels formed with potato infusion plus glucose (dextrose), and malt, two basal media commonly used with molds and yeasts, respectively.

Potato infusion glucose gel. The potato infusion was prepared by boiling 200 g. of

sliced potatoes in 500 ml. of water for 40 to 50 minutes, recovering the liquid portion by coarse filtration, and making it to one l. with distilled water. Then 10 g. of glucose and 3.5 g. of "Calgon" were added to complete the formulation of the basal medium.

Adjustment of pH, addition and blending of the sodium polypectate, and sterilization were the same as described above for the nutrient gel. Gelation was satisfactory and the rigidity of the gel, judged by sensory comparison with the one made with peptone and beef extract, was somewhat higher.

The relationship between pH and concentration of sodium polypectate for the potato infusion, glucose gel was not the same as that observed with the nutrient peptone, beef extract gel. As shown in Table 1, at pH values of 5.0 and above, the pH change was approximately the same (a drop of 10% on autoclaving). However, at values below 5.0, the pH after sterilization increased from 10 to 30% of the original value as the initial pH of the basal medium was decreased.

Growth of the molds was more rapid on the potato infusion, glucose gel but there was no significant change in pectinolytic activity over that observed with the nutrient gel (Table 2).

Malt powder gel. The basal medium was prepared by dissolving 100 g. of powdered malt (Fleischmann) and 3.5 g. "Calgon" in a liter of water. Then the pH was adjusted, the mixture was heated to boiling, the sodium polypectate was added and blending and sterilization were done as described above. However, with this medium the amount of sodium polypectate added for any desired pH must be slightly less than that required

TABLE 2

Effect of initial pH on the ability of various molds to soften the peptone, beef extract, polypectate gel

Genus and species	Number of isolates	Softening as related to initial pH of medium [1]		Final pH of medium
		pH range	pH optimum	
Paecilomyces varioti	1	3.3	3.3	8.0–8.2
Aspergillus sp.	1			
Fusarium gibosum	2	4.2	4.2	8.8–9.2
Fusarium oxysporum	2			
Fusarium lateritium	3			
Fusarium moniliforme	3			
Oospora sp.	1			
Penicillium chrysogenum	3	3.3–4.2	3.3	8.2–9.5
Penicillium notatum	4			
Fusarium moniliforme	2	3.3–4.2	4.2	8.0–9.5
Fusarium oxysporum	1			
Fusarium lateritium	1	3.3–5.2	3.3	8.8–9.0
Penicillium chrysogenum	1			
Fusarium oxysporum	3	3.3–5.2	4.2	8.5–9.2
Fusarium solani	2			
Fusarium roseum	4	4.2–5.2	4.2	9.0–9.2
Streptomyces sp.[2]	1	negative	negative	8.0
Verticillium sp.	1			

[1] The criterion of softening was liquefaction. The pH range indicates the values between which liquefaction occurs. The pH optimum was the value at which liquefaction occurred most rapidly with most extensive involvement of the gel.

[2] The pectinolytic activity of the *Streptomyces* and *Verticillium* cultures was not clearly established. The *Streptomyces* isolate softened the potato infusion gel whereas the unidentified *Verticillium* caused no apparent change in either gel. However, both isolates utilize Wichmann pectic acid as a sole source of carbon in a mineral medium with inorganic nitrogen. Therefore, it cannot be concluded without further study whether these isolates have pectinolytic properties.

for either of the two media already described, except at the lowest pH value. This relationship is shown in Table 1. Satisfactory gels were obtained at all pH values tested.

The pectinolytic yeast *Saccharomyces fragilis (10, 12)* grew well on this malt gel medium and caused its softening at pH values below 5.0, with an optimum pH for softening at about 4.0. Therefore, it appears that if traditional media are indicated for use with the sodium polypectate gel, this latter medium should be useful for rapid demonstration of pectinolysis by *S. fragilis* and, perhaps, other yeasts.

Gels selective for gram negative bacteria. Gram negative nonsporulating bacteria frequently produce pectinolytic enzymes. It was thought that a pectate gel selective for these bacteria might be useful, particularly for rapid detection of plant pathogens and soft rot organisms (of dubious pathogenicity) known to be involved in softening of harvested plant tissues. Two of the more promising of these selective gels were devised by drastic alteration of the two common coliform media, Endo agar and eosine methylene blue agar, as modified by Levine *(8, 9)*. The basic ingredients per liter follow:

Fuchsin sulfite (Endo) gel		*Eosine methylene blue (Levine) gel*	
Bacto-peptone	10 g.	Bacto-peptone	10 g.
Dipotassium phosphate	3.5 g.	Dipotassium phosphate	2 g.
Sodium sulfite	2.5 g.	Bacto-eosine Y	0.4 g.
Bacto-basic fuchsin	0.4 g.	Bacto-methylene blue	0.065 g.
Calgon	3.5 g.	Calgon	3.5 g.

Either gel was prepared by adding the specified concentration of ingredients to distilled water and heating the mixture to boiling. Then sodium polypectate was added (66 g. per liter or, more conveniently, 33 g. per 500 ml.) and mixed with the other constituents in a laboratory blendor. After thorough blending, the mixture was deaerated, either by vacuum or by use of a boiling water bath to remove the air occluded during preparation of the medium. This step is advisable to avoid undue frothing and "blowing" of cotton plugs during sterilization of either selective medium and also is useful in preparation of the other gels). The pH values of these two selective gels ranged between 6.8 and 6.9 after sterilization at 15 p.s.i.g. for 15 minutes.

Cultures of *E. caratovora* and other unidentified gram negative bacteria isolated from freshly harvested coffee cherries, cucumbers and olives grew well in either medium. The *E. caratovora* isolates always caused crateriform depressions in the surface of either gel in petri dish or slant cultures. In contrast, with many other as yet unidentified cultures from various sources, abundant gas production and extensive softening were observed in petri dish or slant cultures. Control cultures of *Escherichia coli* and *Aerobacter aerogenes* grew well but did not cause softening of either gel. *B. macerans* and *B. polymyxa* failed to grow on the fuchsin sulfite or eosine methylene blue gels although they caused extensive liquefaction of the nutrient gel.

Lactose in either gel caused a delay in liquefaction in proportion to the amount of lactose present. Even 0.1% lactose caused a 1 to 2 day delay in appearance of liquefaction with active pectinolytic cultures.

DISCUSSION AND RECOMMENDATIONS

Polypectate gel media described here have been useful for detection of pectinolysis by bacteria, molds, and the yeast *S. fragilis*. It is suggested by the present authors that even better and more specific media may be developed for this purpose. However, there are problems that must be given consideration.

On the basis of present experience the peptone, beef extract gel should be used at or near neutrality for detection of pectinolytic bacteria. The medium should be used under anaerobic as well as aerobic conditions for both types of bacteria may be pectinolytic. The medium selected for the molds should be used at two pH values, about 3.3 and 4.2, respectively. Similar results were obtained with the peptone, beef extract and the potato infusion, glucose gel

media. The advantage of the latter medium may be that it supports more rapid growth of the molds. The powdered malt gel for the yeasts should be used at about pH 4.0 to 4.2, based on our present knowledge. Pectinolytic yeasts apparently occur infrequently in cucumber brines *(5)*. Little is known of their occurrence in olives and other fermenting plant tissues except for figs *(10, 12)* and possibly cocoa beans and cranberries *(10)*. Routine use of the malt gel might contribute to our knowledge of the ecology of such yeasts.

These media can be used for collection of either qualitative or quantitative data as already described by Vaughn *et al. (16)* for aerobic forms. However, with strictly anaerobic bacteria it is preferable to use a "stab" culture which is inoculated in the depths of the medium with a pasteur pipette. It is suggested that Veillon tubes be used. They should be long enough to provide for a gel at least 8 cm. in depth. For best results the inoculated gel should be stratified with at least 1 cm. of "vaspar". The strictly anaerobic bacteria (*C. felsineum* types) as well as the facultative aerobic bacilli (the *B. macerans-B. polymyxa* group) cause gas formation in the medium as well as liquefaction of the gel. Quantitative results with anaerobic and facultative forms can be obtained by "estimation by dilution" or by the "most probable number" techniques already used with the aerobic forms *(16)*.

These pectate media should not be used for purification of pectinolytic cultures. The softening of the gel causes colonies to run together thus making it nearly impossble to obtain pure cultures of bacteria, molds or yeasts.

Pectin degradation (softening) in plant tissues can result from the activity of the intrinsic pectinolytic enzymes produced by the plants or be caused by the pectinolytic enzymes produced by bacteria, molds or yeasts; by adverse chemical and physical treatments; or by various combinations of these different causes. However, these causes, when unrelated to the course of events which take place in a specific outbreak of softening of cucumbers, olives, peppers, *etc.*, only result in perpetuation of confusion of facts by the author(s) and reader(s). Therefore, unless the complete history of the outbreak is known and the pectinolytic enzymes or microorganisms isolated have been shown to cause softening of previously sterilized samples *(13)* similar to those under investigation, conclusions as to the etiology are not warranted. Thus it is clear that *in vitro* demonstration of pectinolysis with these pectate media is only a start in the accumulation of the proof required.

SUMMARY

The use of sodium polypectate gel media for detection of pectinolytic microorganisms was described in some detail. Conventional basal media plus 3.5 g. of "Calgon" per l. were used for preparation of these gels: nutrient peptone beef extract for miscellaneous bacteria; selective media for gram negative bacteria; potato infusion, glucose for molds; and powdered malt for the yeast *S. fragilis*.

It was possible to adjust each of the media to any desired pH value between 3.3 and 7.0. Variations in preparation that were encountered were described. Recommendations were made for the routine use of these pectate gel media for detection of pectinolytic bacteria, molds, and yeasts. Limitations of the media were emphasized.

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A NEW TREATMENT OF HYGROSCOPIC EQUILIBRIA: APPLICATION TO WALNUTS *(JUGLANS REGIA)* AND OTHER FOODS [a]

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Hygroscopic equilibrium data have been obtained for a wide variety of materials, because the derived moisture sorption isotherms serve a number of useful functions. They may be employed: to define limits for the dehydration of fruits, vegetables, nuts, etc. *(28);* to estimate moisture content changes under any given condition of temperature and humidity *(28);* to evaluate processing variables and to distinguish differences between grades or varieties of agricultural commodities *(23, 27);* to aid in the selection of packaging materials *(23, 28);* and to define moisture or humidity conditions under which product deterioration *(2, 6, 10, 19)* and microbial growth can be inhibited *(5, 38, 39)*. In addition, water sorption isotherms of more homogeneous materials, such as natural and synthetic fibres, proteins and other purified materials, can be used in the determination of various physical constants *(1, 7, 11, 12, 13, 16, 18, 24, 29, 36, 40)* and to provide information on the mechanisms of moisture binding *(2* to *4, 9, 21, 29* to *35, 41, 46)*. Diversity in the shapes and amplitudes of the sigmoid moisture sorption isotherms is due to the relative amounts and individual moisture affinities of hygroscopic constituents in the whole material. It would be advantageous to describe moisture sorption isotherms precisely by means of a general mathematical expression, since a minimum amount of experimental data would be required to construct an isotherm and it would permit precise and convenient interpolation of available data. In addition, it would permit the extrapolation to low and especially high humidities.

Hygroscopic equilibrium data are difficult to obtain experimentally at extreme humidities because of the limitations of available hygrometers or because of the rapid deterioration and development of mold at high humidities. Several equations have been derived on empirical or theoretical bases for the description of sorption phenomena and tested against observed data *(2, 3, 4, 7, 8, 12, 16, 18, 21, 29, 36)*. However, previous treatments were only partially successful in that they did not represent the observed isotherms over the complete relative humidity interval, or they exhibited major deviations from observed isotherms or did not adequately represent the sorption isotherms for particular hygroscopic materials. No attempt will be made to consider the extensive literature on this subject, since it has been reviewed in the work cited above and other publications referred to therein.

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The purpose of the work described here was: to obtain hygroscopic equilibrium data for both in-shell and shelled walnuts; to derive a useful mathematical expression for the representation of moisture sorption isotherms of walnuts and other products; and to demonstrate a possible use of moisture sorption isotherms for estimating the relative stabilities of walnuts and other agricultural commodities during storage at various moisture levels.

EXPERIMENTAL

Walnut samples.

1. Franquette variety, "green" in-shell walnuts, 1956 crop, Lake County, California. The whole nuts were ground in a motor-driven meat grinder to pass through a 3/16-inch orifice.
2. Placentia Perfection variety, bleached, in-shell walnuts, 1955 crop, Ventura County, California. After moisture adjustment portions of whole nuts were ground as described for Sample 1.
3. Placentia Perfection variety, light colored, shelled half kernels, hand-cracked and -sorted, 1954 crop, Ventura County, California.
4. Same as Sample 3. Half kernels were cut to 1/16-inch thicknesses with a peanut slicer.

Moisture adjustment. Moisture levels of both shelled and in-shell walnuts were adjusted at a constant temperature of 21° C. in a controlled humidity and temperature conditioning cabinet (Figure 1). To obtain relative humidities below 15%, the cabinet was assisted by the circulation of dry air obtained from a recycling silica-gel air dryer shown at the base of the cabinet. Equilibration of samples to the desired moisture content was usually complete within 48 hours for all samples except those equilibrated at relative humidities below 20% and for which dehumidification periods of up to 5 days were required.

Moisture analysis. A Cenco Moisture Balance,[b] equipped with a 125-watt, industrial infrared lamp, was employed for the estimation of moisture in both shelled and whole-ground shelled walnuts. A voltmeter, variable transformer, and a constant-voltage transformer were added to the electrical circuit to permit reproducible regulation of a two-stage drying process. A two-stage drying process was found to yield more precise results than single-stage drying. The applied voltages were determined empirically from studies on both single- and two-stage drying at various applied voltages. At low voltages, the drying times were prolonged, while scorching occurred at higher voltages.

Sliced, shelled walnuts were dried for 6 minutes at 65 volts, followed immediately by a 9-minute drying period at 100 volts. A moisture reading was taken after the 9-minute period and again at each of two or more 1-minute intervals to ascertain the completeness of drying. The moisture content was taken as the reading obtained after the 9-minute period if no significant change occurred during each of the two 1-minute intervals. If a change of 0.05% moisture was observed during any of the 1-minute intervals, the moisture readings were continued at 1-minute intervals until a difference of less than 0.05% was observed between two successive readings. Moisture was determined in whole-ground, in-shell walnuts in a similar fashion using heating periods of 12 and 18 minutes at 65 and 100 volts respectively, followed by two or more final check readings at 2-minute intervals. The reproducibility of the procedure for whole-ground, in-shell walnuts may be estimated from the data presented in Table 1. A comparison of the results obtained on shelled walnuts by the Moisture Balance procedure, two vacuum oven methods, a Moisture Register and a Karl Fischer titration procedure (Table 2), shows that the moisture values obtained for sliced kernels by the Moisture Balance, the Karl Fischer and the vacuum oven procedure were in good agreement. However, the vacuum oven procedure gave slightly lower values when kernel halves and pieces were used as shown in Tables 2 and 3.

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[b] The mention of special instruments or materials does not imply endorsement by the Department of Agriculture over others of similar nature.

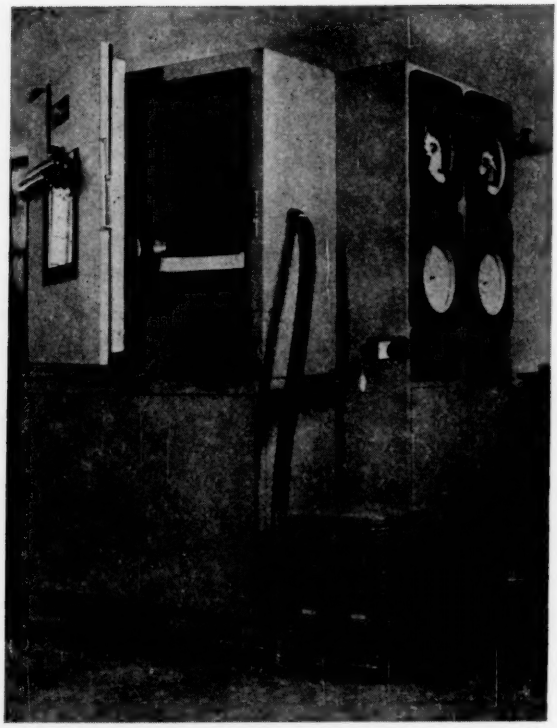


Figure 1. Humidity and temperature controlled moisture-conditioning cabinet. Auxiliary silica-gel air drying apparatus at lower right.

Relative humidity estimation. A direct-reading, A. C. type Aminco-Dunmore electric hygrometer [b] equipped with a multi-element humidity (10% to 99%) and temperature (40° to 100° F.) sensing unit was employed for estimating relative humidity and temperature. The sensing unit was mounted on a stainless steel support containing a magnetically driven fan located below the base of the sensing unit (Figures 2a, b). A rubber gasket was attached below the upper flange to insure a hermetic seal when the sensing unit was mounted in a 1-gallon, wide-mouth jar containing the sample (Figure 3). The fan was rotated by means of gears connected to a vertical shaft which was coupled magnetically to a variable-speed external motor (Figure 4). In actual operation the jar was immersed in a constant temperature water bath. To minimize localized heating within the jar due to frictional forces in the gears and bearings, the fan was operated intermittently by means of a recycling timer. The importance of internal, mechanical convection and the effectiveness of this mechanism in effecting rapid equilibration is indicated in Figure 5. When the fan was operated on a cycle of 1 minute on, 2 minutes off, the rate of equilibration was from 10 to 30 times the rate of an identical sample equilibrated without internal, mechanical convection. Relative humidity values approaching within 1% and 2% of the ultimate value were obtained within 2 and 1 hours, respectively, when mechanical convection was employed, whereas 25 hours were required to approach within 3% of the ultimate value without mechanical convection. In normal operation it was convenient to operate the fan on a cycle of 15 seconds on, 5 minutes off (1 : 20) and to permit samples to equilibrate over night or for periods of not less than 12 hours.

The temperature sensing element was calibrated against a standard glass thermometer

TABLE 1.

Estimate of the precision of the Moisture Balance for the determination of moisture in sliced, shelled and ground, in-shell Placentia Perfection walnuts [1]

Sample	Condition	Replicate determinations	Moisture, range	Moisture, average	Standard deviation,[2] σ	P.E.[3] (N = 3)	V[4]
			%	%	%	%	%
Shelled[5]	Sliced	9	2.45 to 2.70	2.55	0.08	0.03	3.2
In-shell[6]	Ground (3/16 in.)	15	7.60 to 8.20	7.89	0.21	0.08	2.6

[1] Wet basis.

[2] $\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}$.

[3] Probable error $= \frac{0.6745}{\sqrt{N}}\ \sigma$.

[4] Coefficient of variation $= \frac{100\ \sigma}{\text{Average}} = V$.

[5] Light-colored halves and pieces, 1952 crop.

[6] Bleached, commercial sample, 1955 crop.

calibrated by the National Bureau of Standards. The humidity sensing elements were calibrated against a series of saturated salt solutions which are described in Table 4. Reagent grade salts were employed without recrystallization. Observed, corrected, relative humidity values for various other saturated salt solutions at 22.5° C. are shown in Table 5.

Hygroscopic equilibrium data obtained for Placentia Perfection shelled walnut halves and pieces at 7° C. and 22.5° C. and a similar sample of sliced, shelled walnuts at 22.5° C. are presented in Table 6. Moisture desorption equilibrium data for "green", whole-ground, in-shell Franquette variety walnuts as well as adsorption equilibrium data for both whole and whole-ground, bleached, dried Placentia Perfection walnuts are given in Table 7. The analyses of three grades of each of three varieties (Franquette, Payne and

TABLE 2

Comparison of various procedures employed for the estimation of moisture in shelled walnuts [1]

Sample	Procedure					
	Vacuum oven[2]	Vacuum oven[3]	Moisture balance[4]	Moisture register[5]	Karl Fischer[6]	Average
	%	%	%	%	%	%
A	2.35	2.23	2.55	2.00	2.45	2.32
B	3.35	3.25	3.52	3.21	3.45	3.36
C	4.20	4.02	4.30	4.19	4.25	4.19

[1] Placentia Perfection variety, 1952 crop. Each value represents the average of two or more replicate determinations calculated on a wet basis.

[2] Sliced kernels. A.O.A.C. vacuum oven procedure. Methods of analysis of A.O.A.C., 1950 Edition (25.3, page 425). Performed by E. A. McComb.

[3] 25 gm. samples of halves and pieces. Vacuum oven for 72 hrs. at 60° C. and in 27-inch vacuum.

[4] 5 gm. samples of sliced kernels. Moisture Balance procedure as described in text.

[5] Unweighed, approximately 30 gm. samples of halves and pieces at 2,000 pounds pressure. Calibrated against vacuum oven procedure.[3] Model G-5 Moisture Register.

[6] Sliced kernels extracted for 20 min. with formamide, cooled and titrated with Karl Fischer reagent. Analyses performed by E. A. McComb.

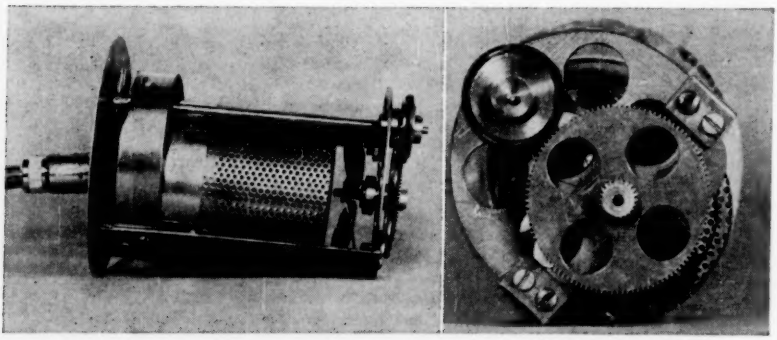
TABLE 3

Comparison of Moisture Balance and vacuum oven procedures for the estimation of moisture in shelled walnuts [1]

Sample	Moisture Balance[2]	Vacuum oven[3]	Difference
	%	%	%
A	0.60	0.04	0.54
B	1.10	0.79	0.31
C	1.62	0.82	0.80
D	1.68	1.42	0.26
E	2.38	1.95	0.43
F	2.55	2.23	0.32
G	2.97	2.27	0.70
H	3.00	2.50	0.50
I	3.12	2.72	0.40
J	3.22	2.93	0.29
K	3.37	2.86	0.51
L	3.45	3.01	0.44
M	3.52	3.24	0.28
N	3.75	3.28	0.47
O	3.80	3.33	0.47
P	3.87	3.38	0.49
Q	4.02	3.65	0.37
R	4.30	4.02	0.28
S	4.32	4.10	0.22
T	4.58	4.58	0.00
U	4.88	4.66	0.22
V	5.13	4.79	0.34
W	5.15	4.98	0.17
Average difference			0.38

[1] Placentia Perfection walnut kernels, 1952 crop.
[2] Sliced kernels. Procedure described in text.
[3] Halves and pieces. 72 hrs. at 60° C., 27-in. vacuum.

Eureka) of shelled walnut halves and pieces and corresponding hygroscopic equilibrium data at three relative humidities are presented in Table 8.



Figures 2a, b. Electronic humidity and temperature sensing head coupled to a magnetically operated, gear-driven fan. a—Side view. b—Bottom view.

TABLE 4

Saturated salt solutions employed for the calibration of the Electric Hygrometer

Salt	Temperature (° C.)	Reference	Relative humidity	
			Literature value	Employed by author
			%	%
Lithium chloride (LiCl)	20	44	11	
	25	44	12	
	22.5			11.5
Magnesium chloride ($MgCl_2 . 6H_2O$)	20	15, 44	33, 33.1	
	25	44	33	
	22.5			33.0
Sodium chloride (NaCl)	20	15	75.8	
	25	44	75	
	30	15	75.1	
	22.5			75.2
Potassium nitrate (KNO_3)	20	15, 44	94, 94.2	
	25	44	93	
	30	15	92.5	
	22.5			93.5

RESULTS

It is generally recognized that the stability of foods and other agricultural commodities is related to their moisture content. In a study on the quantitative relationship between the stability and moisture content of walnuts at moisture levels ranging from 1.5% to 6.0% (wet basis) an unexpected result was obtained. It was observed that there was an apparent optimum moisture level for shelled walnuts, above and below which darkening and the development of rancid flavors and odors occurred more rapidly during

TABLE 5

Relative humidities of some saturated salt solutions at 22.5° C. determined with an Electric Hygrometer

Salt	Author	Literature		
	Relative humidity observed at 22.5° C.[1]	Relative humidity cited	Temperature ° C.	Reference
	%	%		
Potassium acetate ($KC_2H_3O_2 . 1\frac{1}{2} H_2O$)	26.0	22	25	47
Calcium chloride ($CaCl_2 . 6 H_2O$)	31.1	32.3	20	17
Calcium nitrate ($CaNO_3 . 4 H_2O$)	51.8	51	24.5	17
Sodium bromide ($NaBr . 2 H_2O$)	60.0	59	20	39
Ammonium sulphate ($(NH_4)_2SO_4$)	79.7	81	25	17
Potassium chromate (K_2CrO_4)	85.0	88	20	15
Barium chloride ($BaCl_2 . 2 H_2O$)	88.7	88	24.5	17
Di-sodium phosphate ($Na_2HPO_4 . 12 H_2O$)	95.7	95	20	17
Potassium sulphate (K_2SO_4)	95.3	97	20	39

[1] Corrected on the basis of standard salt solutions (Table 4) as described in text.

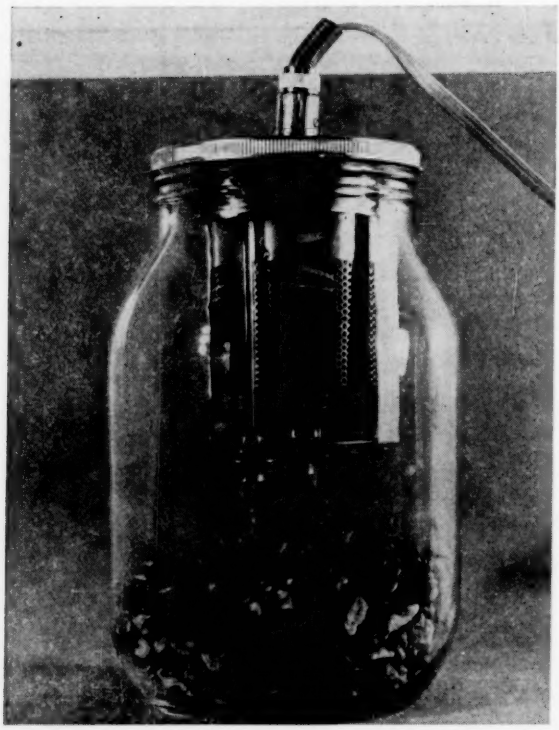


Figure 3. Electronic humidity and temperature sensing head mounted over walnut sample in a one-gallon, wide-mouth jar.

storage at ambient and elevated temperatures *(42, 43)*. More recently it has been observed that when different grades and varieties of shelled walnuts were equilibrated at constant relative humidity, the moisture content of the various groups of kernels varied over a considerable range as shown in the last three columns in Table 8. It may also be seen that the lipid content of the different kernels varied over a relatively wide range (59.8% to 73.0%). Although the data were not entirely consistent, the moisture content of the kernels tended to increase as the hydrophobic lipid content decreased. These observations suggested that the stability of walnuts may be related more closely to their equilibrium relative humidities than to their total moisture contents. In consideration of the small, critical, optimum moisture range for shelled walnuts, it appeared important to establish precise moisture sorption isotherms as the basis for further investigations on factors affecting the stability of shelled walnuts during storage.

Henderson's *(19)* empirical equation (I) for

$$\frac{\ln (1-rh)}{-k\,T} = M^n \qquad (I)$$

rh = equilibrium relative humidity, expressed as a decimal
M = equilibrium moisture content, dry basis, per cent
k = factor, varying with material
n = exponent, varying with material
T = temperature, absolute Fahrenheit (Rankine)

the mathematical description of moisture sorption isotherms suggested the possibility

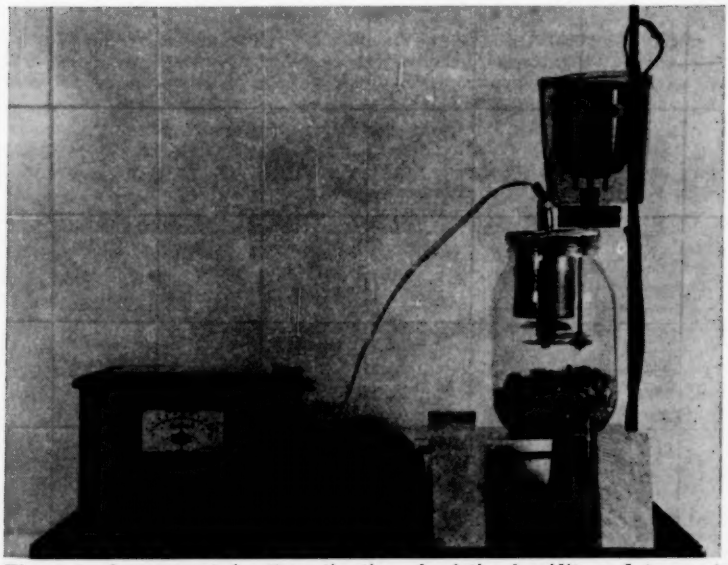


Figure 4. Arrangement for the estimation of relative humidity and temperature inside a hermetically sealed glass jar. The variable-speed, magnetically coupled stirring motor is positioned over an internal bar magnet which rotates the fan blade through the geared vertical shaft. The constant temperature bath and recycling timer are not shown.

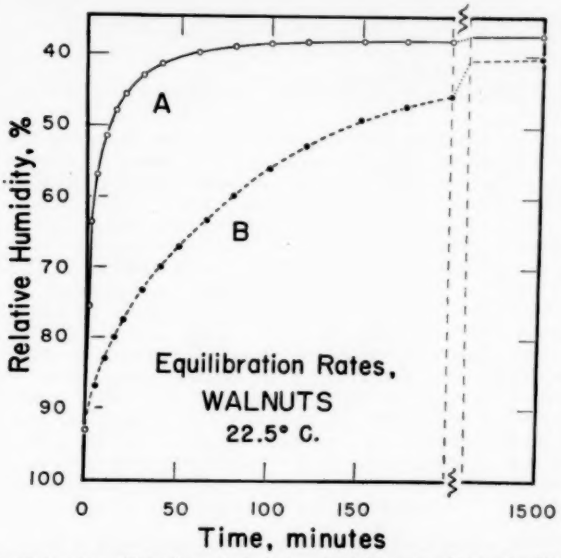


Figure 5. Rate of equilibration of shelled walnut kernels with and without mechanical convection. A. Intermittent mechanical convection, 1 minute on, 2 minutes off. B. Natural convection.

TABLE 6

Relationship between moisture content and equilibrium relative humidity of Placentia Perfection walnut kernels at two temperatures

Moisture content[1] (wet basis)	Walnut halves and pieces[2]		Sliced kernels[3]	Sorption process
	7.0° C.	22.5° C.	22.5° C.	
	Relative humidity	Relative humidity	Relative humidity	
%	%	%	%	
2.35	15.9	13.5		Desorption
2.64	20.2	20.5		
2.88			32.0	
2.95	33.0	36.0	36.4	
3.48			49.1	
3.58	56.6	62.4		
3.78	54.9	63.5	51.8	
3.80	55.0			
3.95	57.0	62.4		
4.08			57.5	
4.50	67.6	71.5		Adsorption
4.67	68.9	78.1		
5.03		80.2		
5.12	74.1			
5.18	77.3	81.0		
5.92		82.0		
6.00		86.2		
6.39	79.6	88.0		
6.50	81.9	86.5		
6.66	87.5	88.1		

[1] Moisture Balance procedure.
[2] Commercial grade light colored kernels.
[3] Commercial grade light colored kernels sliced with a peanut slicer to 1/16-inch thickness.

that the hygroscopic equilibrium data that had been obtained for walnuts might be refined as well as extended to other temperatures. Henderson evaluated the two constants, n and k on the basis of two experimental points, arbitrarily chosen at about 20% and 75% relative humidity. He applied his equation to a series of 18 miscellaneous hygroscopic products. For most of these satisfactory agreement was obtained between the derived theoretical curves and experimental observations from which the constants were evaluated. However, the observed experimental data deviated significantly from calculated theoretical curves for shelled corn, sorghum and other products particularly at extreme relative humidities. Karel *et al. (25)* applied Henderson's equation to the calculation of theoretical curves for shredded coconut, starch, shelled rice, gelatin dessert and flaked oats. These authors also observed significant deviations between the derived experimental curves and observed experimental data particularly at the extreme ends of the relative humidity scale, and offered the suggestion that more than two experimental points be employed for the evaluation of the constants. The present author attempted to apply Henderson's procedure to the hygroscopic equilibrium data obtained for shelled walnuts and obtained essentially the same results as Karel *et al.* Accepting the latter authors' suggestion that a greater number of experimental points be employed in the evaluation of the constants n and k, Henderson's (I) equation was converted to equation (II), a form in which the left

$$\text{(II)} \qquad \log \ln \frac{1}{1-rh} - \log T = n \log M + \log k$$

hand term of the equation should be lineraly related to log M if the equation is valid over the complete relative humidity interval. In equation (II), n represents the slope of the

curve and *log k* is the intercept at *log M* equals zero. Using equation (II) it was possible to evaluate the two constants conveniently on the basis of all experimental points either by graphical extrapolation or by employing the method of least squares. Constants were evaluated for a large number of hygroscopic materials, each at two or more temperatures. The anomalous variation of the constants with temperature did not support the use of the temperature function T in equation (I). Therefore, the temperature term (T) was eliminated, leaving a rearranged form of one of Henderson's earlier equations. For convenience in making computations, the left hand term of the equation was converted from the Naperian log base to the log of the base 10 and the letter Y was substituted for *log k − log 2.303* giving equation (III), which was employed throughout the remainder of the investigations.

$$\text{(III)} \qquad \log \log \frac{1}{1-\text{rh}} = \text{n} \log \text{M} + \text{Y}$$

A plot of $\log \log \frac{1}{1-\text{rh}}$ vs. log M for the data obtained for shelled walnuts yielded

TABLE 7

Relationship between moisture content and equilibrium relative humidity of whole Franquette and Placentia Perfection walnuts at 22.5° C.

Moisture content[1] (*wet basis*)	Green, unbleached whole-ground,[2] *Desorption* Relative humidity	Dried, bleached whole-ground,[3] *Adsorption* Relative humidity	Dried, bleached whole nuts,[4] *Adsorption* Relative humidity
%	%	%	%
3.19	15.0	.....	.....
4.23	.....	30.2	27.2
6.68	41.5	.....	.....
6.96	46.7	.....	.....
7.26	48.2	.....	.....
7.80	.....	60.2	60.2
8.19	.....	61.2	61.2
8.58	.....	65.4	65.8
8.99	.....	73.8	74.0
9.47	.....	76.4	74.6
9.91	67.4	.....	.....
10.40	75.2	.....	.....
10.53	76.2	.....	.....
10.80	72.9	.....	.....
11.39	.....	80.3	79.9
11.43	79.3	.....	.....
11.57	79.8	.....	.....
11.68	77.6	.....	.....
11.92	80.0	.....	.....
12.25	78.3	.....	.....
15.08	91.0	.....	.....
15.55	91.3	.....	.....
15.67	94.1	.....	.....
18.88	97.5	.....	.....
21.05	97.8	.....	.....
29.08	98.7	.....	.....
38.75	99.6	.....	.....

[1] Moisture Balance procedure on whole nuts ground to pass through 3/16 in. orifice on motor-driven meat grinder.
[2] Fresh, undried, unbleached Franquette walnuts from 1956 crop, ground as described in[1].
[3] Dried, bleached Placentia Perfection walnuts from 1955 crop, ground as described in[1]. Original moisture of bleached nuts was 4.2%.
[4] Same as[3] using whole, in-shell nuts.

TABLE 8

Analyses of three grades of Franquette, Payne, and Eureka varieties of shelled walnuts [1]

Variety	Color grade	Moisture, original[2]	Color[3] units	Lipid[4]	Protein[5]	Carbohydrate,[6] fiber and ash	Moisture at various relative humidities[7]		
							12[8]	30[2]	46[2]
		%		%	%	%	%	%	%
Franquette	Light	4.15	21	73.0	16.8	10.2	2.08	3.08	3.68
	Medium	4.10	39	62.0	.....	.....	1.89	3.10	3.88
	Dark	4.10	63	60.0	17.6	22.4	2.13	3.30	3.95
Payne	Light	4.12	18	66.2	17.9	15.9	1.68	3.12	3.55
	Medium	4.30	37	65.9	.....	.....	1.82	3.25	3.65
	Dark	4.32	71	66.2	19.9	13.9	2.10	3.95	4.32
Eureka	Light	4.22	26	68.3	16.8	14.9	1.90	3.02	3.65
	Medium	4.30	52	59.8	.....	.....	1.78	3.35	3.88
	Dark	4.30	84	63.5	16.0	20.5	1.85	3.46	3.98

[1] Shelled halves and pieces, machine-cracked, hand-sorted, 1953 crops. Obtained through the courtesy of the Diamond Walnuts Growers, Stockton, Calif.
[2] Moisture Balance procedure as described in text.
[3] Units per 100 gm. of shelled kernels using procedure described by Rockland *et al.* (43).
[4] Total lipid, calculated to a moisture-free basis, based on moisture values at 12% relative humidity.
[5] Nitrogen x 6.25. Moisture-free basis, based on moisture at 12% relative humidity.
[6] Total dry weight minus the sum of the protein and lipid.
[7] Brought to constant moisture at specified relative humidities and 70° F.
[8] Karl Fischer titration using formamide as extraction solvent.

an unanticipated result as shown in Figure 6. It may be seen that two intersecting lines rather than a single straight line are suggested. Similar plots using hygroscopic equilibrium data for over 50 other materials yielded similar results except that certain data yielded as many as three apparent intersecting lines as shown for salmine, gelatin and gelatin dessert in Figures 7 and 8. It was concluded that the equation is not valid over the complete relative humidity range when a single pair of constants, n and Y, are employed for the derivation of a theoretical curve. These results further suggested that moisture sorption may not necessarily be a uniform, continuous process, but a series of two or more independent processes each of which could be characterized in terms of equation (III) if suitable constants, calculated from data corresponding to the "localized isotherms", were substituted in the equation. A complete moisture sorption isotherm over the entire range would be composed of two or more coupled, intersecting "localized isotherms", each representing a limited relative humidity interval. The construction of theoretical isotherms in this manner for over 50 miscellaneous hygroscopic materials supported this premise. Nearly perfect agreement was observed between theoretical curves and all reliable experimental hygroscopic equilibrium data. An example of the construction of a sorption isotherm by coupling two "localized isotherms" is shown for shelled walnut kernels in Figure 9. The agreement between the total theoretical isotherm and the original, mixed adsorption and desorption hygroscopic equilibrium data is indicated in Figure 10. Additional examples of theoretical isotherms, derived from coupled, "localized isotherms" for salmine, gelatin, potatoes, beans, flaked oats, gelatin dessert and carrots are shown in Figures 11 to 14. It may be seen that when pure adsorption or pure desorption data are employed for the evaluation of "localized isotherm" constants, and therefrom the calculation of the theoretical isotherm, the latter is found to fit the observed data with a high degree of precision over the complete relative humidity scale. The possible usefulness of this technique in obtaining complete moisture sorption isotherms from limited experimental data is reflected in the sorption isotherm for Great Northern beans, shown in Figure 12. In this figure, the large open circles represent samples of beans which had become moldy before complete equilibration with the saturated salt over which they were suspended. Therefore, these points cannot be considered as reliable points

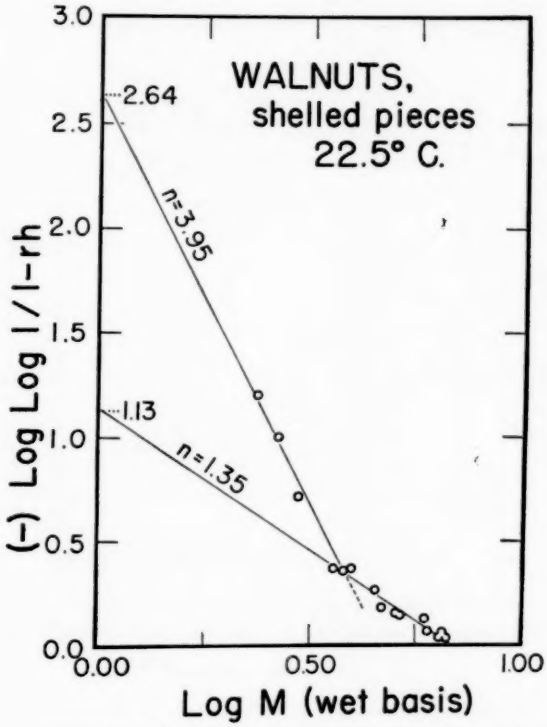


Figure 6. Plot of log log $\left(\frac{1}{1-rh}\right)$ vs. log moisture, for shelled walnuts at 22.5° C., showing values of constants Y and n for the two apparent straight line curves.

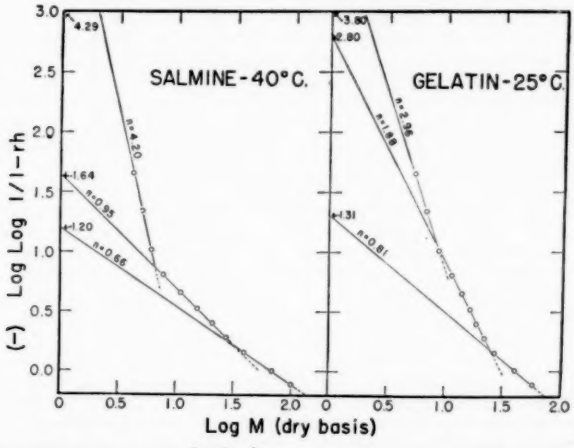


Figure 7. Plots of log log $\left(\frac{1}{1-rh}\right)$ vs. log moisture for Bull's hydroscopic equilibrium data for Salmine at 40° C. and gelatin at 25° C. (8) showing constants Y and n for each of three curves for each protein.

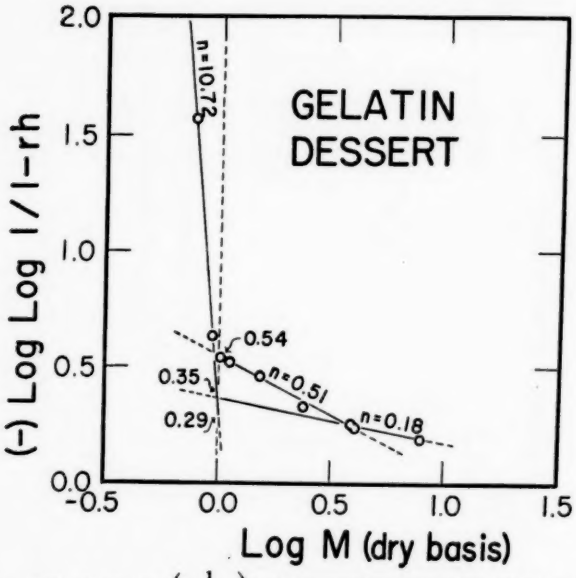


Figure 8. Plot of log log $\left(\frac{1}{1-rh}\right)$ vs. log moisture for Karel et al.'s (25) hygroscopic equilibrium data for Gelatin Dessert, showing the constants Y and n for three curves.

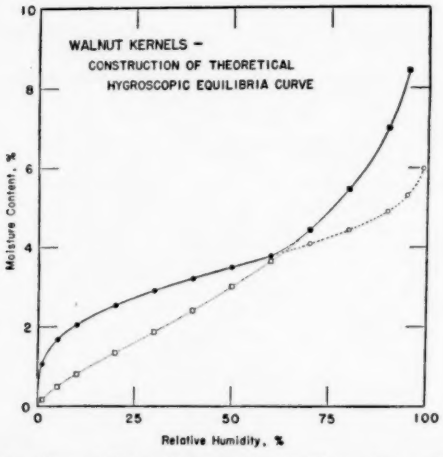


Figure 9. Construction of theoretical sorption isotherm for walnut kernels. Circles represent theoretical points derived by substituting the Part 1 constants n_1 and Y_1, in equation III. Squares represent theoretical points derived by substituting Part 2 constants, n_2 and Y_2, in equation III. The solid curve represents the final theoretical isotherm.

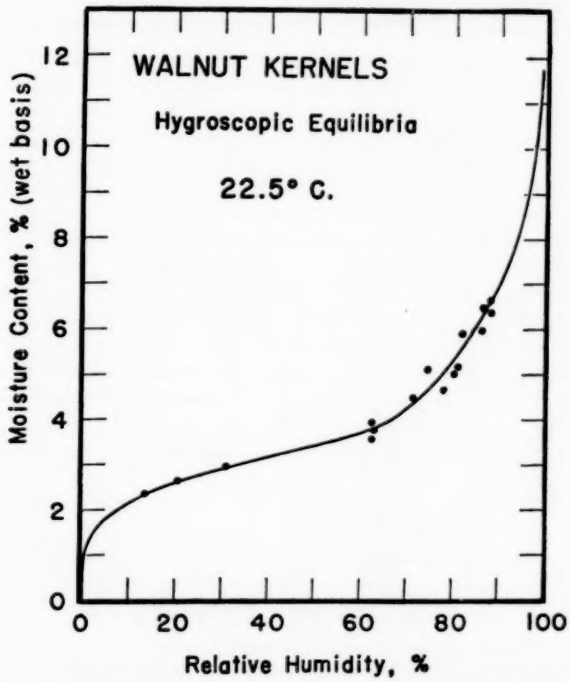


Figure 10. Comparison of theoretical sorption isotherm with observed hygroscopic equilibrium data for shelled walnut kernels at 22.5° C. Solid line, theoretical curve. Solid circles, observed data.

on the isotherm. The theoretical portion of the isotherm shown above the large circles was based on the constants calculated for the same "localized isotherm" using experimental data at lower relative humidities where the development of mold did not occur before equilibration was complete. While experience with isotherms of similar products does not suggest the presence of a fourth "localized isotherm", which might modify the shape of the extrapolated region of the curve, the reliability of the extrapolation cannot be ascertained without experimental verification.

Theoretical sorption isotherms for shelled walnuts at 7° C. and 22.5° C. are shown in Figure 15. A theoretical desorption isotherm for "green", in-shell walnuts and a theoretical adsorption isotherm for bleached, in-shell walnuts, each at 22.5° C. are presented in Figure 16. These isotherms were calculated from the data given in Tables 6 and 7 and their corresponding constants, calculated by the method of least squares, presented in Table 9. The same table lists constants, calculated similarly from data provided in the literature, for 40 natural products including: purified salmine and gelatin; whole Spanish and Runner peanuts; Runner peanut kernels, meats, hearts, skins and shells; Pinto, Great Northern and Michelite beans; rough, white and parboiled Rexora rice; brown Caloro rice; long grain, quick-cooking rice; starch; flaked oats; shredded cocoanut; gelatin dessert; unblanched Savoy cabbage, Prickly Winter spinach, Chantenay carrots, Russet white potatoes, and Puerto Rico yams. It may be noted in Table 9 that isotherm constants have been calculated on either a wet or a dry basis depending upon the literature data from which the constants were derived. For theoretical purposes it would be preferable to employ a dry basis especially for purified materials such as gelatin and salmine. However, for food products, such as walnuts, peanuts and beans, it is

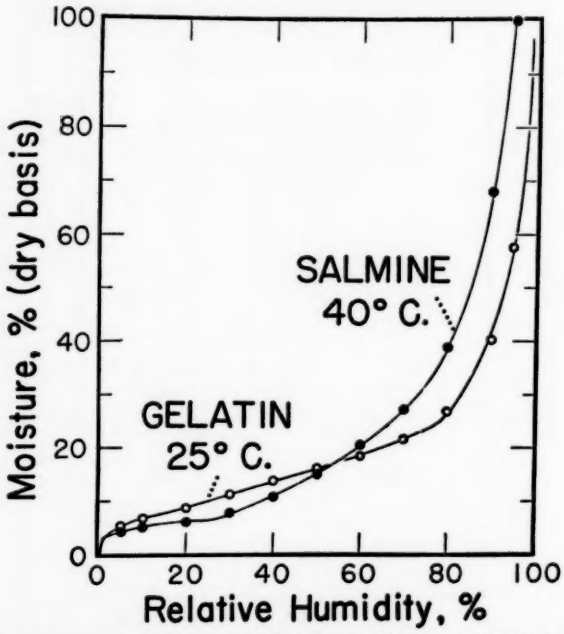


Figure 11. Comparison of theoretical adsorption isotherms (solid lines) with Bull's (8) observed hygroscopic equilibrium data for Salmine (solid circles) at 40° and Gelatin (open circles) at 25° C.

often more convenient to consider moisture levels on a wet basis. The intercepts of the "localized isotherms" along the moisture axis may be calculated from equation (IV) where the subscript s refers to local isotherm 1, 2, etc.

$$\log M_{i\,(s,\,s+1)} = \frac{Y_{s+1} - Y_s}{n_s - n_{s+1}} \qquad \text{(IV)}$$

The intercepts of the "localized isotherms" along the relative humidity axis may be calculated from equation (V).

$$\left[\log \log \frac{1}{1-\mathrm{rh}}\right]_{(z,\,z+1)} = n_s \log M_{i\,(s,\,s+1)} + Y_s \qquad \text{(V)}$$

Satisfactory estimates of theoretical moisture sorption isotherms at intermediate temperatures have been obtained using interpolated values of the isotherm constants within a range of 10° C.

DISCUSSION

Hailwood and Horrobin *(16)*, McLaren and Rowen *(29)*, Windle *(46)* and other workers cited by these authors have suggested that the binding of water by polymers is a multi-stage process involving at least two and according to some workers three states of water. It is of interest that for a large number of hygroscopic materials, a plot of $\log \log \frac{1}{1-\mathrm{rh}}$ vs. $\log M$ yields at least two and most generally three straight lines (Figures 6, 7, and 8) each of which represents the moisture sorption over a limited relative humidity

interval. A future publication is planned for the presentation of added evidence in support of the "localized isotherm" hypothesis. For this reason and because the derivation of useful moisture sorption isotherms is based on an empirical process, further theoretical discussions will be minimized.

For the present purposes it has been assumed that a complete moisture sorption isotherm is composed of coupled, independent "localized isotherms" each representing a limited relative humidity interval. If this assumption is valid, it might be concluded that the total isotherm would not necessarily be a smooth, continuous function. Although most published isotherms appear to be smooth, sigmoid curves, examples of apparently discontinuous isotherms have been reported. Excellent examples of the latter are found in Glaeser and Mering's *(14)* adsorption isotherms for Ca-Na montmorillonites. Smooth, apparently continuous moisture sorption isotherms, such as those reported by Henderson *(19)* for cotton, spray dried eggs, etc., are not opposed to the concept of coupled "localized isotherms". Although it is possible that a complete isotherm may be represented by a single pair of constants in equation

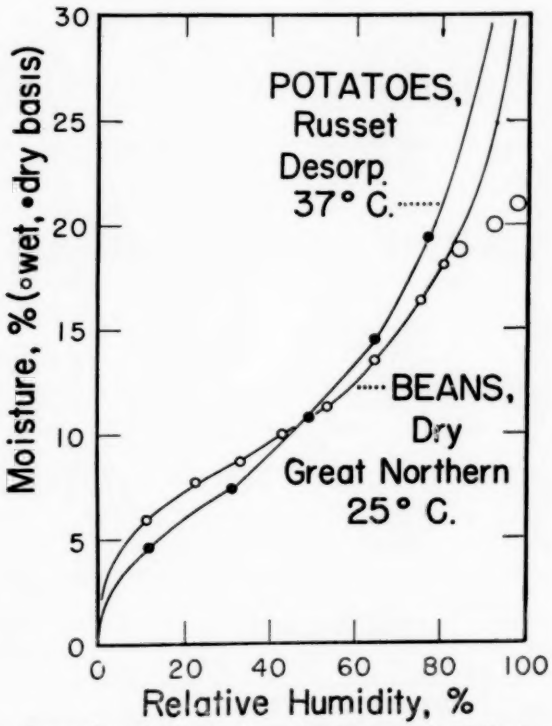


Figure 12. Comparison of theoretical sorption isotherms (solid lines) with Makower and Dehority's (28) observed desorption data for white Russet potatoes at 37° C. (solid circles) and Weston and Morris' (44) data for Great Northern beans at 25° C. (small open circles). The large open circles represent pre-equilibrium values obtained when first mold growth was observed on the bean samples.

TABLE 9

Moisture sorption isotherm constants, n and Y, for walnuts, various food materials and other natural products [1]

Isotherm No.	Source of data	Material	Sample	Variety	Condition	Moisture basis	Initial moisture	Sorption process	Temperature degrees C.	Hygrometric method	Isotherm constants					
											Part 1		Part 2		Part 3	
											n_1	Y_1	n_2	Y_2	n_3	Y_3
							%									
1	Author	Walnuts, in-shell	1	Franquette	Fresh, ground	Wet	ca 35	Desorption	22.5	Electric hygrometer	1.77	−2.05	0.56	−0.52	....	.....
2	Author	Walnuts, in-shell	2	Placentia	Bleached, ground	Wet	4.2	Adsorption	22.5	Electric hygrometer	1.60	−1.81	....	.....	....	.....
3	Author	Walnut, kernels	3	Placentia	Pieces	Wet	4.2	Combined	7.0	Electric hygrometer	3.28	−2.34	1.48	−1.30	....	.....
4	Author	Walnut, kernels	3	Placentia	Pieces	Wet	4.2	Combined	22.5	Electric hygrometer	3.96	−2.64	1.35	−1.13	....	.....
5	Author	Walnut, kernels	4	Placentia	Sliced	Wet	4.2	Desorption	22.5	Electric hygrometer	2.18	−1.75	....	.....	....	.....
6	8	Salmine				Dry		Adsorption	25.0	Salt solutions	3.98	−4.24	0.99	−1.70	0.59	−1.09
7	8	Salmine				Dry		Adsorption	40.0	Salt solutions	4.20	−4.29	0.95	−1.64	0.66	−1.20
8	8	Gelatin				Dry		Adsorption	25.0	Salt solutions	2.96	−3.80	1.88	−2.80	0.81	−1.31
9	8	Gelatin				Dry		Adsorption	40.0	Salt solutions	....	.....	1.91	−2.79	0.94	−1.48
10	27	Peanuts, whole	280	Spanish	Fresh, whole	Wet	29.93	Desorption	25.0	Salt solutions	2.63	−2.72	1.38	−1.62	0.82	−0.98
11	27	Peanuts, whole	227	Spanish	Cured, whole	Wet	6.91	Combined	25.0	Salt solutions	2.62	−2.71	1.39	−1.61	0.53	−0.65
12	27	Peanuts, whole	228	Spanish	Dried, whole	Wet	5.96	Combined	25.0	Salt solutions	2.77	−2.83	1.45	−1.68	0.53	−0.65
13	27	Peanuts, whole	261	Runner	Dried, whole	Wet	6.80	Combined	25.0	Salt solutions	2.61	−2.73	1.40	−1.63	0.91	−1.12
14	27	Peanuts, whole	262	Runner	Dried, whole	Wet	6.63	Combined	25.0	Salt solutions	2.63	−2.73	1.40	−1.64	0.71	−0.87
15	27	Peanuts, whole	263	Runner	Cured	Wet	6.21	Combined	25.0	Salt solutions	2.41	−2.59	1.48	−1.74	0.75	−0.92
16	27	Peanuts, whole	263	Runner	Cured, re-wet	Wet	14.5	Desorption	25.0	Salt solutions	2.55	−2.71	1.58	−1.86	0.63	−0.76
17	27	Peanuts, whole	263	Runner	Cured, re-dried	Wet	1.5	Adsorption	25.0	Salt solutions	2.31	−2.39	1.64	−1.86	0.60	−0.72
18	27	Peanut kernels	263	Runner		Wet		Combined	25.0	Salt solutions	3.16	−2.89	1.62	−1.75	0.69	−0.60
19	27	Peanut meats	263	Runner		Wet		Combined	25.0	Salt solutions	3.06	−2.78	1.20	−1.34	0.56	−0.66
20	27	Peanut hearts	263	Runner		Wet		Combined	25.0	Salt solutions	3.35	−3.19	1.57	−1.78	0.69	−0.93
21	27	Peanut skins	263	Runner		Wet		Combined	25.0	Salt solutions	2.94	−3.90	1.01	−1.37	....	.....
22	27	Peanut shells	263	Runner		Wet		Combined	25.0	Salt solutions	2.15	−2.72	....	.....	....	.....
23	44	Beans		Pinto		Wet		Combined	25.0	Salt solutions	3.37	−3.96	2.62	−3.24	1.51	−2.07
24	44	Beans		Great Northern No. 16		Wet		Combined	25.0	Salt solutions	3.12	−3.71	2.47	−3.08	1.55	−2.10
25	44	Beans		Michelite No. 78		Wet		Combined	25.0	Salt solutions	3.11	−3.17	2.41	−3.03	1.50	−2.05
26	26	Rice, rough		Rexora		Wet			25.0	Salt solutions	2.27	−2.83	....	.....	....	.....
27	22	Rice, brown		Caloro		Wet	14.8	Combined	25.0	Salt solutions	2.73	−3.48	....	.....	....	.....
28	26	Rice, white		Rexora		Wet	15.6	Combined	25.0	Salt solutions	2.56	−3.28	....	.....	....	.....
29	23	Rice, parboiled		Rexora		Wet	13.1	Combined	25.0	Salt solutions	2.60	−3.35	1.94	−2.48	....	.....
30	23	Rice, quick-cooking		Long-grain		Wet	9.5	Combined	25.0	Salt solutions	2.90	−3.51	1.35	−1.76	....	.....
31	25	Rice				Dry				Electric hygrometer	1.73	−2.17	....	.....	....	.....
32	25	Oats, flaked				Dry				Electric hygrometer	4.76	−5.39	2.68	−3.34	....	.....
33	25	Starch				Dry				Electric hygrometer	3.60	−4.38	2.24	−3.03	....	.....
34	25	Cocoanut			Shredded	Dry				Electric hygrometer	0.83	−0.84	0.46	−0.62	....	.....
35	25	Gelatin dessert				Dry				Electric hygrometer	10.72	−0.29	0.51	−0.54	....	.....
36	28	Cabbage	Unblanched	Savoy	Fresh leaf	Dry	>30.8	Desorption	37.0	Salt solutions	1.67	−1.97	0.70	−1.24	1.45	−1.77
37	28	Cabbage	Unblanched	Savoy	Dried leaf	Dry	< 2.3	Adsorption	37.0	Salt solutions	1.45	−1.77	0.70	−1.23	....	.....
38	28	Spinach	Unblanched	Prickly Winter	Fresh leaf	Dry	>27.2	Desorption	37.0	Salt solutions	2.41	−2.68	1.00	−1.52	0.57	−1.02
39	28	Spinach	Unblanched	Prickly Winter	Dried leaf	Dry	< 3.4	Adsorption	37.0	Salt solutions	2.42	−2.53	0.87	−1.35	0.78	−1.25
40	28	Carrots	Unblanched	Chantenay	Sliced, fresh	Dry	>32.9	Desorption	37.0	Salt solutions	2.26	−2.20	0.78	−1.38	....	.....
41	28	Carrots	Unblanched	Chantenay	Sliced, dried	Dry	< 0.7	Adsorption	37.0	Salt solutions	1.00	−1.42	0.74	−1.31	....	.....
42	28	White potatoes	Unblanched	Russet	Sliced, fresh	Dry	>19.4	Desorption	37.0	Salt solutions	2.34	−2.82	1.53	−2.12	....	.....
43	28	White potatoes	Unblanched	Russet	Sliced, dried	Dry	< 4.4	Adsorption	37.0	Salt solutions	2.25	−2.73	1.65	−2.24	....	.....
44	28	Yams	Unblanched	Puerto Rico	Sliced, fresh	Dry	>20.5	Desorption	37.0	Salt solutions	2.11	−2.31	0.91	−1.37	....	.....
45	28	Yams	Unblanched	Puerto Rico	Sliced, dried	Dry	< 3.5	Adsorption	37.0	Salt solutions	1.98	−2.35	0.83	−1.28	....	.....

[1] Calculated by method of least squares.

(III), it has been observed in studies of more than 75 hygroscopic materials that with few exceptions, at least two and usually three "localized isotherms" may be distinguished. Complete moisture sorption isotherms would appear as smooth, continuous sigmoid curves if the constants for the "localized isotherms" are reasonably close together. It has been observed that when one "localized isotherm" dominates the total isotherm in the intermediate relative humidity interval, "localized isotherms" may not be distinguished readily at the extreme ends of the scale. In addition, deviations from smooth, sigmoid curves would tend to be overlooked at low relative humidities because of uncertainty or unavailability of hygroscopic equilibrium data in this range. Without exception, substitution of reliable, raw, wide-range hygroscopic equilibrium data in equation (III) suggested the presence of at least two and generally three "localized isotherms". Single component isotherms for various rice and walnut samples are listed in Table 9. However, no attempt was made to obtain complete moisture sorption isotherms for all walnut samples, and the data presented in the table for certain samples of walnuts are considered incomplete. It cannot be determined from the available data whether or not the unprocessed rice samples have single components, whether they are composed of multiple components that cannot be distinguished or whether local isotherms may occur at the extreme ends of the relative humidity

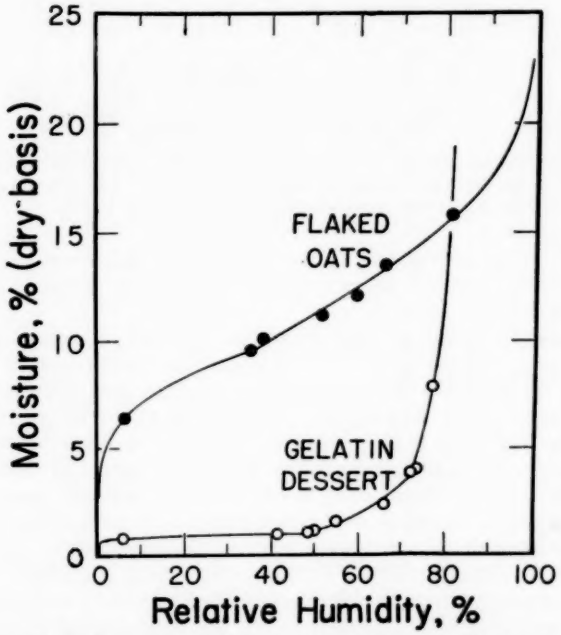


Figure 13. Comparison of theoretical sorption isotherms (solid lines) with Karel, Aikawa and Proctor's (25) observed hygroscopic equilibrium data for flaked oats (solid circles) and gelatin dessert (open circles).

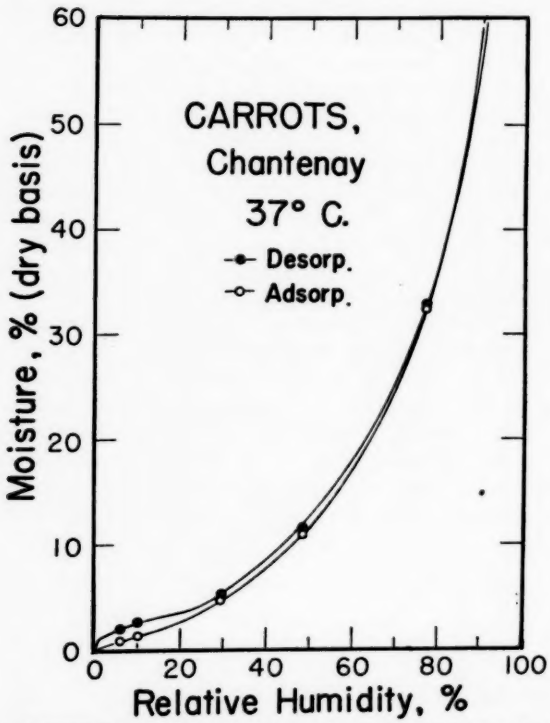


Figure 14. Comparison of theoretical desorption and adsorption isotherms (solid lines) with Makower and Dehority's (28) desorption (solid circles) and adsorption (open circles) hygroscopic equilibrium data for Chantenay carrots at 37° C.

scale. It is of interest that a second component appears in the isotherms of processed rice products as indicated in Table 9 for parboiled and quick-cooking rice.

The observation *(42, 43)* that walnut kernels have an optimum moisture range above and below which darkening and the development of off-flavors occur at a more rapid rate suggests that at least two types of chemical processes are involved. The rates of development of free fatty acids and one type of pellicle darkening are associated with high moisture levels, whereas a second type of pellicle darkening and the development of rancid odors are promoted at moisture levels below the optimum. Oyxgen absorption, peroxide formation and the evolution of carbonyl compounds are accelerated on both sides of the optimum moisture range, the most pronounced changes occurring at moisture levels above 5.5%. On the premise that a fraction of the total moisture content is bound to proteins, carbohydrates, etc., and therefore may be relatively unavailable as a reactant, it appeared logical to consider the deterioration process in terms of changes in the amount of "active water", defined in this connection as the change in partial pressure of water $(\triangle p)_T$ or relative humidity $(\triangle p/p_o)$, per unit change of total moisture in the kernels.

If "active water" is a primary reactant, it would be expected that finite changes in total moisture would have minimal effects on the deteriorative reactions if the resultant change in the equilibrium relative humidity of the system were also minimal. Conversely, minimal changes in total moisture associated with a significant modification of the equilibrium relative humidity would be expected to strongly influence chemical reactivity. If this premise is correct, it would be reasonable to expect that the moisture sorption isotherm for shelled walnuts would have a region, corresponding closely with the observed optimum stability range, which demonstrates a minimal change in relative humidity per unit change in total moisture content. Therefore, the moisture sorption isotherm for shelled walnuts at 22.5° C. was employed to derive the change in relative humidity per unit change in moisture, or the differential coefficient of relative humidity with respect to moisture ($\triangle$ rh/$\triangle$ M) as a function of moisture content. For the purposes of comparison the reciprocals of these functions ($\triangle$ M/$\triangle$ rh) and observed stability characteristics of shelled walnuts were each plotted against corresponding moisture levels as shown in Figure 17. The similarity between the observed stability curves and the derivatives of the moisture sorption isotherms of walnuts (Figure 17) and Great Northern Beans (Figure 18), respectively, is apparent.

The double minima shown in the stability curves for both walnuts and

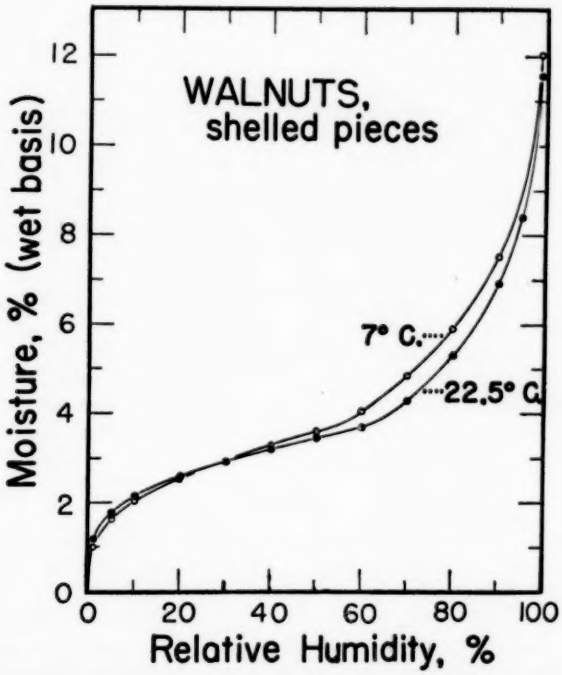


Figure 15. Theoretical sorption isotherms for Placentia Perfection shelled walnut halves and pieces at 7° C. (open circles) and 22.5° C. (solid circles).

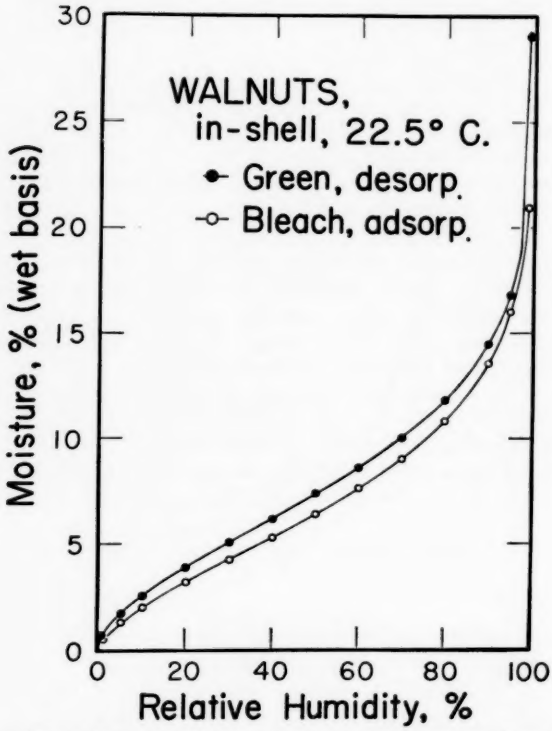


Figure 16. Theoretical desorption isotherm for "green", whole-ground, in-shell Franquette walnuts (solid circles) and a theoretical adsorption isotherm for bleached whole or whole-ground, in-shell Placentia Perfection walnuts at 22.5° C.

beans may have special significance in respect to the nature of their moisture sorption isotherms. It is of interest that the $\triangle M/\triangle rh$ curves for both products which also exhibit double minima, were derived from theoretical moisture sorption isotherms constructed from multiple, "localized isotherms". It is apparent that only a single minimum would have been obtained for each product if their respective moisture sorption isotherms are smooth sigmoid curves containing a single inflection point.

SUMMARY

Procedures have been developed for the rapid estimation of moisture in both shelled and in-shell walnuts. A magnetically operated fan has been combined with the sensing unit of an electric hygrometer to provide mechanical convection and hence rapid attainment of moisture—moisture vapor equilibrium of samples in a hermetically sealed container. The importance of mechanical convection in facilitating rapid equilibration and the estimation of equilibrium relative humidity in shelled walnuts has been demonstrated. Moisture sorption isotherms have been determined for various samples of both in-shell and shelled walnuts.

It has been suggested that moisture sorption isotherms are composed of multiple, but most generally three "localized isotherms", each of which may represent a special type of water binding. Each "localized isotherm" can be distinguished by graphical analysis of experimental data plotted according to a simplified form of the equation derived by Henderson. Each "localized isotherm" is characterized by a pair of constants, n and Y, which can be obtained by graphical interpolation or more precisely by the method of least squares. Theoretical "localized isotherms" are calculated by substituting the derived constants in the original equation. The total moisture sorption isotherm is obtained by coupling the portion of the individual "localized isotherms" which corresponds to the portion of the relative humidity scale for which each of the "localized isotherms" is valid. Moisture sorption isotherms derived in this manner fit the observed data with a high degree of precision and can be employed for reliable interpolation and extrapolation of available data. "Localized isotherm" constants have been presented for 45 natural products including: shelled and in-shell walnuts; purified salmine and gelatin; peanuts, peanut kernels, meats, hearts, skins and shells; beans;

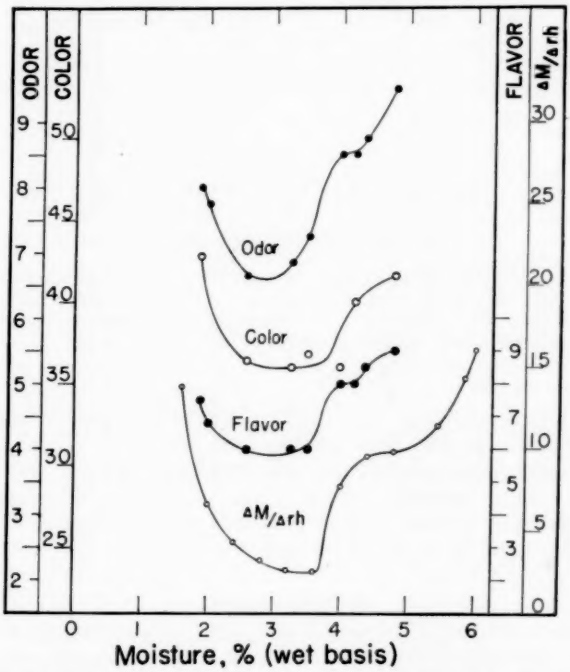


Figure 17. Comparison of $\triangle M/\triangle rh$ curve (small, open circles) for Placentia Perfection walnuts at 22.5° C. with observed values for flavor, color and odor of shelled Placentia Perfection walnuts after storage in sealed glass jars for 7 months at 35° C. Flavor and odor values obtained by a trained panel using a hedonic scale of 1 (excellent) to 10 (very rancid). Color was evaluated by the procedure of Rockland, Slowdoski and Luchsinger (43).

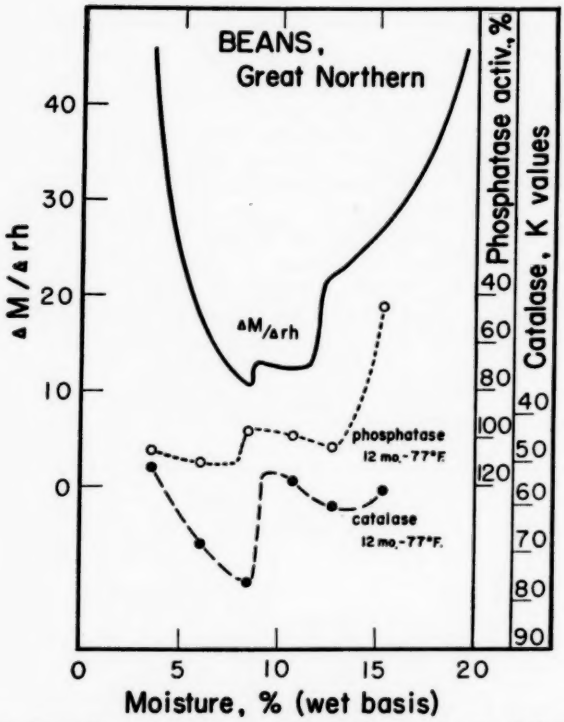


Figure 18. Comparison of $\triangle M/\triangle rh$ curve (solid curve) for Great Northern beans at 25° C. (77° F.), calculated from its theoretical sorption isotherm (Figure 12), with Morris and Wood's (37) data on the relative activities of phosphatase (open circles) and catalase (closed circles) in Great Northern beans after storage for 12 months at 77° F.

rice and rice products; flaked oats; starch; shredded coconut; gelatin dessert; cabbage; spinach; carrots; white potatoes; and yams.

It has been suggested that the differential coefficient of moisture with respect to relative humidity ($\triangle M/\triangle rh$), which can be calculated from moisture sorption isotherms, may be related to stability characteristics of shelled walnuts and beans during storage.

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SOME APPLICATIONS OF RANK ORDER STATISTICS IN SENSORY PANEL TESTING[a]

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In a recent paper on sensory panel testing, Krum *(4)* outlined a test for comparing means of ranks assigned to a set of 'at least 3' samples by a panel of '10 or more' judges. The test is in essence an unpaired t-test which, in his numerical example, was applied to the largest of the 6 possible differences among a set of 4 samples. On the basis of this test, the difference was significant at the 5% level, from which one would conclude that a difference between the two samples had been demonstrated. The conclusion would, however, be unwarranted because the test is biased in two ways, viz., (a) ranking errors are inherently negatively correlated, so that the error variance will tend to be underestimated, and (b) the difference between the largest and the smallest mean in a set of 3 or more means will be larger, on the average, than the difference between a randomly selected pair of means. Both of these factors will tend to increase the relative frequency of significant differences above the 1 in 20 that is allowable for samples that are identical.

The negative correlation between ranking errors is inevitable because when a judge ranks one sample too high he will rank one or more of the other samples too low. If a set of n samples is ranked, the average correlation coefficient for a pair of them can be shown to be $-1/(n-1)$. This will be negligible in a large set of samples, but for a small set it may be appreciable, e.g., for a set of 3 samples, $E(r) = -0.5$. For this value of r, the variance of the difference between a pair of rank means will be understated by 1/3 if Krum's procedure is used.

The second source of bias on the other hand will increase in importance as the number of samples is increased. For uncorrelated, normally distributed variables the difference between the largest and the smallest mean in a set of 4 will, in the long run, yield a significant t value once in about 5 times when the true means are identical. Similarly, the largest difference in a set of 8 means will be significant once in two trials.

TESTING A COMPLETE SET OF 3 OR MORE RANK MEANS

There is, however, a simple test of significance that is specifically designed for a multiple ranking procedure such as this. Developed by Kendall and Babington Smith *(3)*, it consists of a comprehensive test of the mean ranks for the entire set of samples, in much the same way that the well known F test is used to test a set of means in an analysis of variance. The mathematical basis of the test and its application are described in detail by Kendall *(2)*, so the method will be outlined only briefly here.

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Kendall employs the test criterion

$$W = \frac{12\,S}{m^2\,(n^3 - n)}$$

which he calls the coefficient of concordance, where

S = sum of squares of deviations of rank sums from their mean,
n = the number of items being ranked, e.g. the number of treatments,
m = the number of rankings, e.g., the number of judges in the panel.

Tables for testing W are available *(2)* for n = 3, m = 2 to 10; n = 4, m = 2 to 6; n = 5, m = 3. For higher values of n and/or m, an approximate test of W can be made by referring the quantity

$$F = \frac{(m-1)\,W}{1 - W}$$

to tables of F, with degrees of freedom

$$\nu_1 = n - 1 - \frac{2}{m}$$

and

$$\nu_2 = (m-1)\nu_1$$

Although the degrees of freedom will always be fractional, interpolation will seldom be necessary except for occasional borderline F values. In these cases linear interpolation on the reciprocals of ν_1 and ν_2 will, in general, be adequate. When numerous ties occur, the test procedure requires some modification as indicated by Kendall.

For n greater than 7, an even simpler test due to Friedman *(1)* may be employed, since then the quantity

$$m(n-1)\,W$$

is approximately distributed as χ^2 with n − 1 degrees of freedom.

In the cited numerical example *(4)* the rank sums for the four samples were 32, 29, 28 and 21, so that

$$S = 32^2 + 29^2 + 28^2 + 21^2 - \frac{110^2}{4} = 65.$$

There were 11 rankings, so S = 65, m = 11 and n = 4 are substituted in the above formulae to give W = 0.1074 and F = 1.203 with 2.82 and 28.2 degrees of freedom. Even without interpolation it is apparent that F is well below the 5% point, which means that the 4 samples as a group did not differ significantly. This being the case, one cannot justifiably proceed to single out individual pairs of treatments for significance tests, unless the selection is based entirely on *a priori* considerations, without reference to the observed rankings.

For most practical purposes, interest will be confined to a test of significance, i.e., to determining whether the chance probability, P, of obtaining so large a coefficient of concordance is less than 0.05, say. However, if desired, an approximate value of P can be readily obtained from a nomogram prepared by Pearson and Hartley [*(5)*, Table 17]. Only 3 quantities need be calculated, viz., $a = \frac{\nu_2}{2}$, $b = \frac{\nu_1}{2}$ and x = 1 − W. In the above example, a = 14.1,

$b = 1.4$ and $x = 0.893$; for these values $P = 0.32$ is read directly from the nomogram.

The test of concordance described above is an adaptation of the randomization test *(6, 8)*, which does not depend on any assumption about normally distributed errors. The randomization test is similar in its procedure to the 'normal theory' analysis of variance test for a randomized block design, except that the numbers of degrees of freedom are modified on the basis of the observed distribution of intra-block (here intra-judge) variances. If they are more uniform than would be expected for normally distributed errors, the degrees of freedom are diminished; if less uniform, they are increased.

In testing a complete set of n rank means, the intra-block variances are of course identical (provided there are no ties), and this condition is the basis of the concordance test. However, if a subset of rank means is to be tested, the assumption of equal intra-block variances will not be valid, and so the more general form of the randomization test is required. It is described below, with a numerical example.

TESTING A SUBSET OF 3 OR MORE RANK MEANS

In general, if a set of n samples has been ranked by a panel of m judges, a test of a subset of n′ sample means ($3 \leqslant n' < n$) selected from prior considerations can be made as follows. The variance ratio is calculated from the subset just as it would be for the 'normal theory' randomized block analysis with n′ treatments and m blocks, i.e.,

$$F = \frac{(m-1)\,S_T}{S_E},$$

where S_T and S_E are the sums of squares for treatments and for error.

However, in referring this variance ratio to tables of F, the degrees of freedom will not, in general, be $(n'-1)$ and $(m-1)(n'-1)$, but rather

$$\nu_1 = \frac{(n'-1)(m-1)}{m(1-A)} - \frac{2}{m}$$

and

$$\nu_2 = (m-1)\,\nu_1,$$

where A is a heterogeneity factor defined as

$$A = \frac{\sum_1^m a_i^2}{\left(\sum_1^m a_i\right)^2},$$

and a_i is a measure of the variation within the ith block which, for ease of calculation, may be defined as

$$a_i = n' \sum_1^{n'} X^2 - \left(\sum_1^{n'} X\right)^2.$$

When both m and n′ are large, the F test with (n′ − 1) and (m − 1) (n′ − 1) degrees of freedom should ordinarily be sufficiently accurate. However when either m or n′ are less than 5, the degrees of freedom should be calculated as indicated above.

To illustrate the procedure, let us assume that samples 2, 3 and 4 in the above example *(4)* represented 3 'levels' of a certain treatment, and that this treatment was basically different from the one received by sample 1. Then, irrespective of the observed rankings, it might be of interest to determine whether there were significant differences among the rankings of these three samples. The randomization test would accordingly be applied as follows.

Judge No.	X_2	X_3	X_4	ΣX	ΣX^2	$a = 3\Sigma X^2 - (\Sigma X)^2$
1	3	4	1	8	26	14
2	3	4	2	9	29	6
3	4	1	2	7	21	14
4	1	4	3	8	26	14
5	1	3	2	6	14	6
6	1	2	3	6	14	6
7	2	4	1	7	21	14
8	3	2	1	6	14	6
9	4	1	3	8	26	14
10	3	2	1	6	14	6
11	4	1	2	7	21	14
	29	28	21	78		114

For the ranks assigned by Judge No. 1, for example, $\Sigma X = 3 + 4 + 1 = 8$, $\Sigma X^2 = 3^2 + 4^2 + 1^2 = 26$ and $a = 3(26) - 8^2 = 14$.

The heterogeneity factor is

$$A = (14^2 + 6^2 + \ldots + 14^2)/114^2 = 0.1043$$

which, on substitution in the above formulae with m = 11 and n′ = 3, gives 1.85 and 18.5 degrees of freedom for F.

The treatment sum of squares is

$$S_T = \frac{29^2 + 28^2 + 21^2}{11} - \frac{78^2}{33} = 3.455,$$

while the error sum of squares can be calculated directly from the a as follows:

$$S_E = \frac{\sum_{i}^{m} a_i}{n'} - S_T = \frac{114}{3} - 3.455 = 34.545.$$

Since F = 10(3.455)/34.545 = 1.00, there were no appreciable differences among the 3 samples. For F values bordering on significance, linear interpolation on the reciprocals of ν_1 and ν_2 will usually be sufficiently accurate.

TESTING A SUBSET OF 2 RANK MEANS

If n treatments have been ranked by m judges and if a particular pair of these treatments is to be compared, again from considerations independent of the observed rankings, a different procedure is suggested since the randomization test outlined above probably does not provide a satisfactory approximation for n′ as small as 2. For this situation a simple binomial test known as the sign test is outlined below. Like the randomization test, it requires no assumption about the distribution of ranking errors.

If there was no detectable difference between the two samples, A and B, half of the judges would be expected to rank A ahead of B, and half would be expected to rank B ahead of A. In other words, the expected frequency with which each sample will be ranked ahead of the other in m independent ranks is $\frac{m}{2}$. The observed frequency may of course differ from this expected value by pure chance. What is required, therefore, is a test to determine whether the difference is too great to be reasonably attributed to chance.

This test is based on the fact that, in the case of identical samples, the number of A (and of B) preferences is approximately normally distributed about a mean of $\frac{m}{2}$ with a standard deviation of $\frac{\sqrt{m}}{2}$. If the larger of the two observed frequencies is denoted by f, the difference between the samples will be judged significant if $\left(f - \frac{1}{2} \right)$ exceeds $\frac{m}{2}$ by more than twice the standard deviation, i.e., by more than $\sqrt{m}$.

For instance, sample 4 was ranked ahead of sample 3 by 7 of the 11 judges, so that $\left(f - \frac{1}{2} \right) = 6.5$, and $\frac{m}{2} = 5.5$. Since the difference, 1.0, is less than $\sqrt{11}$, no significant difference is indicated.

The 3 test procedures described above belong to a class of statistical techniques known as non-parametric or distribution-free methods. An extensive bibliography on the developments in this field has been prepared by Savage *(7)*.

SUMMARY

Attention is drawn to the dangers of the indiscriminate use of well established statistical procedures. An example is given in which a set of four rank means was judged significantly different at the 0.05 level by the incorrect application of the t-test. What was needed was the W-test, and this is outlined here. When applied to the same data, far from attaining significance, it indicated a chance probability of 0.32.

The principles underlying the W-test are used to develop a simple procedure that can be used for testing a subset of 3 or more rank means, provided the selection of the subset is not influenced by the rankings themselves. For a subset of 2 rank means, the familiar sign test is recommended.

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EFFECT OF FOUR CONDITIONS OF COOKING ON THE EATING QUALITY OF TWO CUTS OF BEEF [a, b]

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Differences among certain basic cooking methods depend upon variations in the temperature and moisture content of the cooking medium. These variables are subject to control in the laboratory. Previous studies in this laboratory have attempted to relate such cooking conditions to those changes in meat which enhance its eating quality *(2, 3, 4, 5, 7)*. The present study is a continuation of this series. It provides the following contrasts: cuts with little and much connective tissue; cooking by moist and dry heat; and cooking to different degrees of doneness as measured by internal temperature.

EXPERIMENTAL

Meat was available from 26 yearling steers of about the same age, produced in 1955 at Substation No. 23 of the Texas Agricultural Experiment Station. They had been fed the same ration under similar conditions. Ten of the carcasses graded U. S. Commercial and 16, U. S. Good. These steers were from various breeds and crosses. No attempt will be made here to assess the relationship of the eating quality of these steers with either production or carcass records.

Steers were slaughtered in the meats laboratory of the Animal Husbandry Department. After storage for 7 days, each carcass was divided into the primal cuts and then into 1-inch steaks. Seven steaks from the anterior end of the loin and 5 or 6 from the dorsal end of the bottom round were obtained from each side of each carcass. They were frozen at minus 20° F. and stored at 0° F. until tests could be made. Loin steaks were trimmed to include the *longissimus dorsi* muscle and adhering fat but no bone or other muscles. Bottom round steaks were trimmed to include the *biceps femoris* muscle and adhering fat but no bone or other muscles.

Cuts 1 through 4, numbered from the anterior end of loin and dorsal end of round, were used for tests of eating quality. Paired cuts from the left and right sides of the same animal were cooked—one by a "moist heat method" (braising) and its pair mate by a "dry heat method" (oven-broiling). Within each of these methods, the meat was cooked to two internal temperatures, selected to provide extremes of doneness by each method of cooking.[c] Thermometers were inserted into the frozen steaks when the temperature was about minus 2° C. and the cooking was started as nearly as possible at 0° C.

Oven-broiling. In previous studies in this laboratory, thin steaks (¾-inch) had been broiled at 200° C. (392° F.) in a gas broiler compartment with the broiler door left open as recommended by Cooperative Meat Investigations *(1)* and by Lowe *(8)*. However, Cover and Shrode *(5)* and Cover and Smith *(7)* found that under these conditions the

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[a] The meat used was from animals raised at Substation 23, McGregor, Texas under a project which is cooperative with the U. S. Department of Agriculture and is a part of S-10 Southern Regional Beef Cattle Breeding Project.

[b] The authors wish to acknowledge the assistance of Dr. M. J. Garber, formerly with the Department of Genetics, for his help with the statistical designs and R. L. Smith, Statistical Laboratory, for making the statistical calculations.

[c] Meat from a few animals had a slight pink color after broiling to 80° C. While pink juice did not run out of the meat or collect on the plate, a tiny amount of pink juice could be pressed out by squeezing with the fingers. The cause of this unusual condition is unknown but it is distinctly an animal variation.

broiler temperature was affected by breezes in the laboratory or by any movement in front of the open broiler door. Doors and windows had to be closed and no fans could be on, even when room temperatures were above 100° F.

Because an experimental method was needed which would be similar to those used in the home yet would have the advantages of easy standardization and because oven temperatures are more easily standardized than broiler temperatures, broiling in a *well-ventilated* gas oven was attempted. In this case the heat transfer appeared to be by radiation from the hot oven walls, by convection from the hot broiler pan as well as from the hot gases from the combustion chamber, and by conduction from the hot broiler rack. When a granite broiler pan was used, the steak browned on the top only and needed to be turned. Preliminary work indicated considerable spattering of fat when steaks were cooked in an oven at 200° C. (392° F.) but much less spattering when the oven temperature was lowered to 175° C. (347° F.). Simone, Carroll, Hinreiner and Clegg *(12)* roasted their steaks at 149° C. (300° F.). This is the low temperature recommended for roasting beef. They considered that roasting afforded the best control and reproducibility. They used top round steaks which weighed between 3 and 4 pounds. Their steaks were not turned and required about one hour to reach an internal temperature of 66° C. (150° F.)—probably medium-rare. Cover and Shrode *(5)* roasted top round cuts of similar weight but called them "roasts" instead of "steaks" because they were 2½-inches thick. Steaks used in the present study were 1-inch thick and weighed ½ to ¾ pound. The loin steaks reached an internal temperature of 61° C. in about 26 minutes.

Broiling was done in well-ventilated gas ovens with the oven doors closed—one steak per oven. Oven and meat thermometers were read through the glass oven door. The oven, with the large blue enameled broiler pan and wire rack in place within it, was regulated at 175° C. (347° F.) before broiling was begun. This temperature is higher than is recommended for roasting beef. Steak temperatures were recorded at 1-minute intervals. Rare steaks were turned at 50° C. and removed at 61° C. The well-done steaks were turned at 65° C. and removed at 80° C. Adequate venting of the oven prevented the accumulation of moisture and the well-done steaks browned nicely. This method of cooking steaks is practical for home use.

Braising. The braised steaks were cooked in steam. A heavy pot was preheated to 475° F. in a standardized manner over a gas flame *(5, 7, 9)*. One tablespoon of cooking oil was added and the steak browned for one minute on each side. After browning, a rack was placed under the steak, hot water was added to a short distance below the rack, and the heavy lid was adjusted. The liquid was brought to a boil quickly, and the fire adjusted to maintain a steady flow of steam through the opening in the lid around the meat thermometer. Steak temperatures were recorded at 1-minute intervals. Only one steak was cooked in each pot. Half of the braised steaks were removed at an internal temperature of 85° C. For the others the cooking was continued to an internal temperature of 100° C. and for 25 minutes thereafter. The steaks which were removed at 85° C. were underdone. The meat was slightly pink and the juice had a pink tinge. The steaks which were cooked to an internal temperature of 100° C. and held there for 25 minutes were well-done. They were used to simulate the conditions in many homes where braising time may be from 45 to 60 minutes.

Eating quality. Cores for shears and samples for the judges were cut as described by Cover and Smith *(7)*. Steaks 1 and 2 from both sides of the same animal provided meat for the 4 conditions of cooking each cut. This made a total of 4 loin and 4 bottom round steaks for one judging session. Another judging session for the same animal was held several weeks later using steaks 3 and 4. This allowed both left and right sides from each cut of each animal to be represented in each method of cooking. Judging sessions were held in the morning and afternoon, 4 days each week. The composition of the judging committee was uniform for all of the animals included in this report. A few more animals were available but were excluded because a judge was absent unexpectedly.

Scores for juiciness for all samples were obtained as in the previous study, but the tenderness scores were obtained in a different manner. Scores for tenderness, reported in the previous study *(7)*, were a composite of all subjective impressions of tenderness or toughness, but it was suggested there that some of the toughness might have been caused by the muscle fiber component of the meat and some by the connective tissue component. In an effort to separate these tenderness components, the tenderness scores

in this study were obtained in two ways. A "connective tissue" score was reported only when obvious connective tissue was identified by sight or detected during mastication. A score for "muscle fibers" was obtained for all samples. It included not only the tenderness of the muscle fibers but also the tenderness of any connective tissue which seemed inseparable from them. Thus, tenderness scores in this study are not directly comparable with those of the previous study.

Statistical treatment of the data. Analyses of variance were calculated using the outlines given in Table 1. Coefficients of correlation were calculated for certain pairs of variables.

TABLE 1

Outline of analyses of variance

	Source of variation	Degrees of freedom	Error terms
Analysis I.	Cuts	1	Animals
	Methods within cuts	6	Animals
	Doneness within broiled loin	(1)	Animals
	Doneness within braised loin	(1)	Animals
	Broiled vs. braised within loin	(1)	Animals
	Doneness within broiled bottom round	(1)	Animals
	Doneness within braised bottom round	(1)	Animals
	Broiled vs. braised within bottom round	(1)	Animals
	Animals within methods and cuts	200	Steaks
	Steaks within animals, methods and cuts	208	
	Total	415	
Analysis II.	Methods	3	Animals
	Cuts within methods	4	Animals
	Loin vs. bottom round within 61° C.	(1)	Animals
	Loin vs. bottom round within 80° C.	(1)	Animals
	Loin vs. bottom round within 85° C.	(1)	Animals
	Loin vs. bottom round within 100° C.+	(1)	Animals
	Animals within cuts and methods	200	Steaks
	Steaks within animals, cuts and methods	208	
	Total	415	
Analysis III.	Cuts	1	Animals
	Methods within cuts	2	Animals
	80° C. vs. 100° C.+ within loin	(1)	Animals
	80° C. vs. 100° C.+ within bottom round	(1)	Animals
	Animals within cuts and methods	100	Steaks
	Steaks within animals, cuts and methods	104	
	Total	207	

RESULTS AND DISCUSSION

Weight of steaks. The weight of the raw steaks averaged about the same for the different conditions of cooking when the steaks were from the same cut (Table 2). Bottom round steaks were considerably heavier than Loin steaks.

Time of cooking. Total time of cooking under the same conditions was somewhat longer for the heavier bottom round steaks than for the loin steaks (Table 2). In broiled steaks, the increase in doneness from rare (61° C.) to well-done (80° C.) involved an increase of only 19° C. in internal temperature, but it more than doubled the total time of cooking in both cuts. This marked change in the rate of heat penetration may be observed in Figure 1. In braised steaks of both cuts, only a slight change in the rate of heat penetra-

TABLE 2

Weights, weight losses and cooking times, averages of two steaks from each of 26 animals

Variables[1]			Weight raw	Total cooking time	Weight loss during cooking
Comparisons by temperatures within methods and cuts					
			(grams)	*(minutes)*	(%)
Loin	Broil	61° C.	205	26	14
(L. dorsi)		80° C.	209	56	35
			n.s.	***	***
	Braise	85° C.	203	11	21
		100° C.+	209	42	35
			n.s.	***	***
Bottom round	Broil	61° C.	303	30	13
(B. femoris)		80° C.	314	75	37
			n.s.	***	***
	Braise	85° C.	298	15	24
		100° C.+	315	50	39
			*	***	***
Comparisons by cuts within temperatures and methods					
Broil	61° C.	Loin	205	26	14
		BR	303	30	13
			***	***	*
	80° C.	Loin	209	56	35
		BR	314	75	37
			***	***	***
Braise	85° C.	Loin	203	11	21
		BR	298	15	24
			***	***	***
	100° C.	Loin	209	42	35
		BR	315	50	39
			***	***	***

[1] The cuts were loin and bottom round (BR). The methods of cooking were broiling to 61° C. and 80° C. and braising to 85° C. and to 100° C. plus 25 minutes.

*, **, *** indicate significance at the 5%, 1%, and 0.1% levels, respectively; n.s., above 5% level.

tion occurred between 85° C. and 100° C. (Figure 2), but the total cooking time was increased purposely in the well-done steaks by a holding period of 25 minutes at 100° C.

Weight losses. In general, weight losses during cooking were slightly higher for bottom round than for loin steaks. Bottom round steaks were considerably heavier and had the longer cooking times. For steaks of the same cut cooked by the same method but to different internal temperatures, cooking losses were considerably greater in the more thoroughly cooked meat within each method. Greater weight losses in the more thoroughly cooked broiled meat must have come from evaporation as well as from drippings (moisture, fat, proteins and salts). In braised steaks, the losses in the more thoroughly cooked meat must have come from drippings only as the cooking

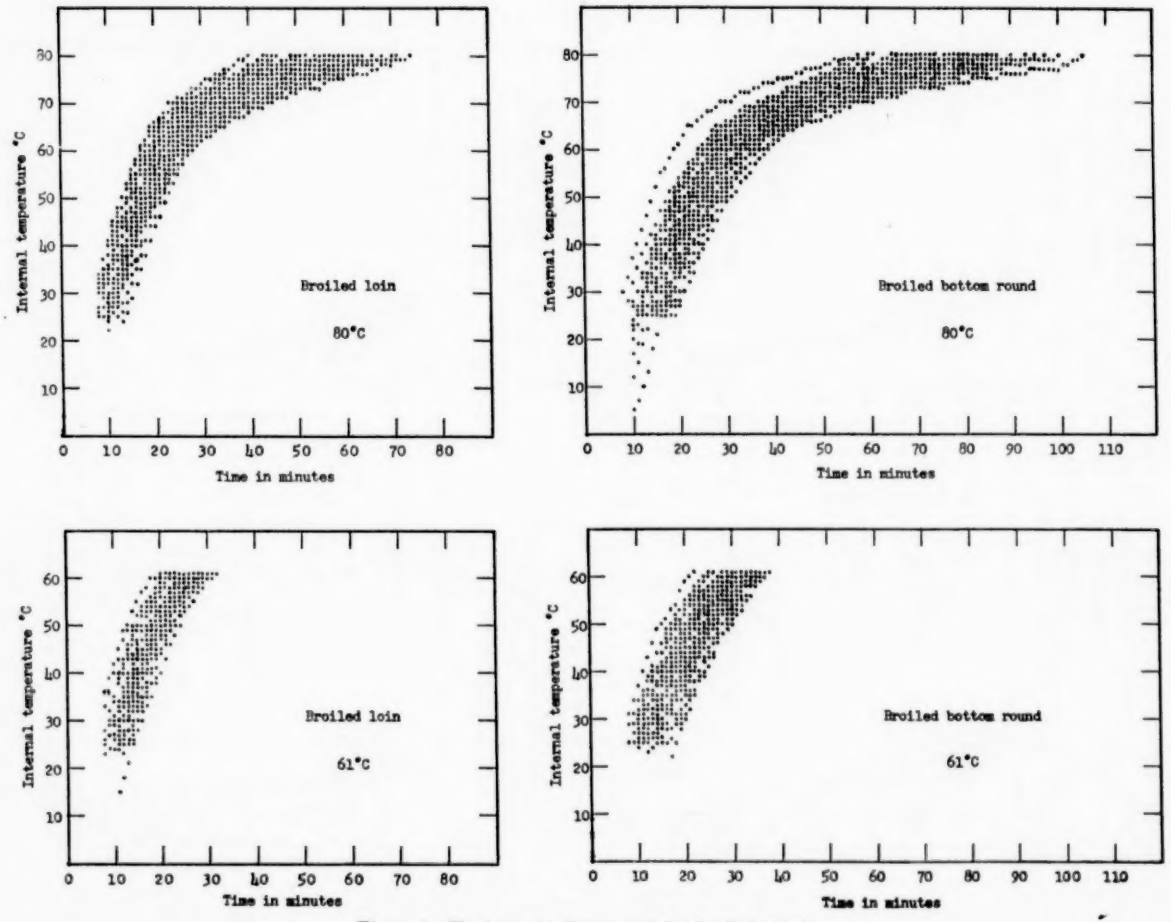

Figure 1. Heat penetration curves for broiled steaks.

medium was saturated with water vapor. Cover and Smith *(6)* reported evidence from thiamine data that the surface of braised meats appeared to have been washed by the condensing steam.

In comparison with the ¾-inch steaks of Cover and Smith *(7)*, these 1-inch loin steaks weer about 40 g. heavier and these bottom round steaks

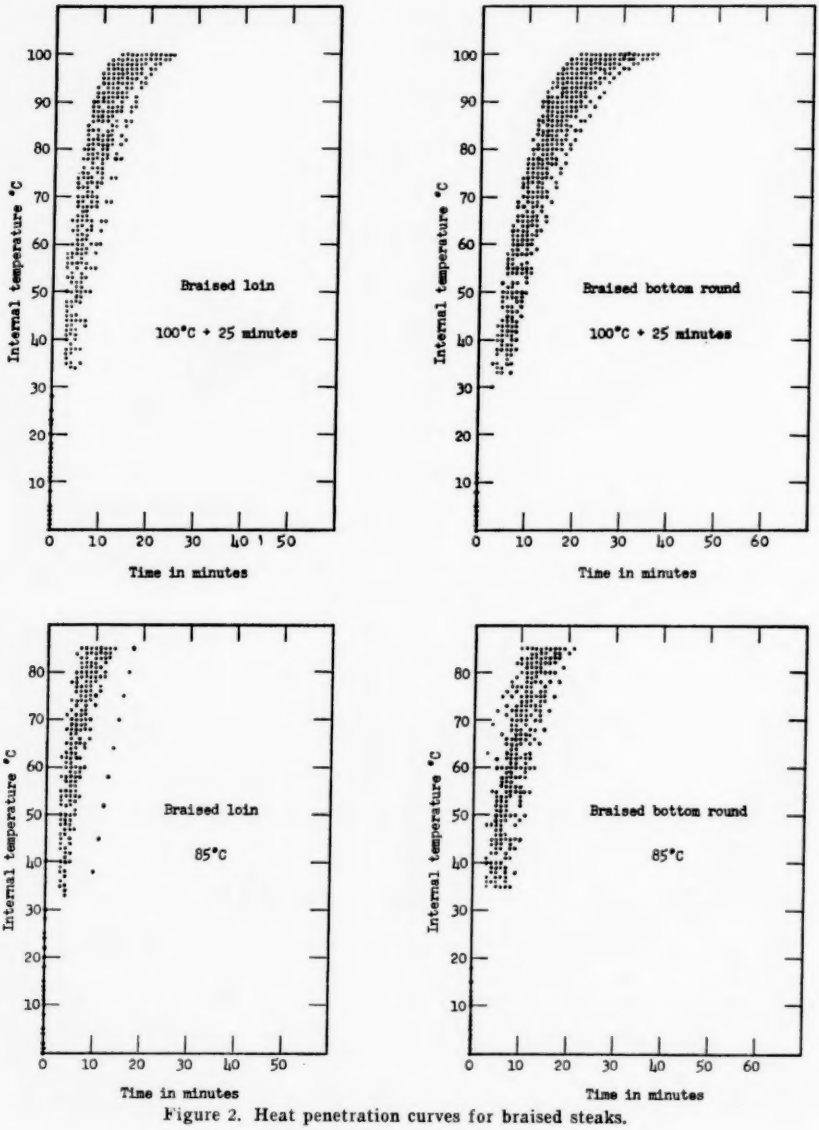

Figure 2. Heat penetration curves for braised steaks.

about 100 g. heavier. Comparisons of cooking times in this study with those of the previous study indicated that the broiling time was somewhat longer for these well-done 1-inch steaks which were oven-broiled to a definite internal temperature but that the braising time did not differ greatly from the 45 minutes used in the former study.

Juiciness scores. Juiciness scores were higher for the less well-done than for the more thoroughly cooked steak within each cut and method of cooking (Table 3). The more juicy steaks also were those cooked for a shorter time

TABLE 3

Juiciness and tenderness scores and shear force values, averages of two steaks from each of 26 animals

Variables[1]			Juiciness scores[2]	Shear force value[3]	Tenderness scores	
					Obvious connective tissue[4]	Muscle fibers[2, 5]
Comparisons by temperatures within methods and cuts						
Loin (*L. dorsi*)	Broil	61° C.	8.7	7.4	5.0 (16)	7.2
		80° C.	5.3	9.8	4.9 (14)	6.0
			***	***		***
	Braise	85° C.	5.8	9.4	5.3 (16)	5.6
		100° C.+	3.3	9.3	7.2 (9)	6.0
			***	n.s.		n.s.
Bottom round (*B. femoris*)	Broil	61° C.	9.2	9.7	2.2 (208)	6.7
		80° C.	5.3	9.2	2.5 (194)	5.3
			***	n.s.		***
	Braise	85° C.	5.3	9.4	2.7 (187)	5.4
		100° C.+	3.4	6.4	4.9 (181)	6.5
			***	***		***
Comparisons by cuts within temperatures and methods						
Broil	61° C.	Loin	8.7	7.4	5.0 (16)	7.2
		BR	9.2	9.7	2.2 (208)	6.7
			***	***		*
	80° C.	Loin	5.3	9.8	4.9 (14)	6.0
		BR	5.3	9.2	2.5 (194)	5.3
			n.s.	n.s.		**
Braise	85° C.	Loin	5.8	9.4	5.3 (16)	5.6
		BR	5.3	9.4	2.7 (187)	5.4
			***	n.s.		n.s.
	100° C.+	Loin	3.3	9.3	7.2 (9)	6.0
		BR	3.4	6.4	4.9 (181)	6.5
			n.s.	***		*

[1] The cuts were loin and bottom round (BR). The methods of cooking were broiling to 61° C. and to 80° C. and braising to 85° C. and to 100° C. plus 25 minutes.

[2] Based on scores from 4 judges for each steak. Scoring by weighted adj.: 0, lowest; 10, highest.

[3] Based on 4 shears for each steak.

[4] Total number of possible judgments was 208. Number of scores on which average is based is given in parentheses.

[5] These scores include also any connective tissue which seemed indistinguishable from the muscle fibers.

*, **, *** indicate significance at the 5%, 1%, 0.1% levels, respectively; n.s., above 5% level.

and those with the lower weight losses within each cut and method of cooking (Tables 2 and 3.

When juiciness scores were compared by cuts within each temperature and method of cooking, only small differences in juiciness occurred between the two cuts. Yet these small differences in juiciness were significant at the 0.1% level in two cases: in favor of the bottom round when broiled to 61° C. but in favor of the loin when braised to 85° C. The cause of these differences was not apparent.

When only well-done steaks were considered, the juiciness scores were considerably higher after broiling than after braising (Table 4). An analysis

TABLE 4

Comparisons of steaks cooked well-done by broiling and braising

Variables			Weight loss during cooking (%)	Juiciness scores	Shear force values *(lb.)*	Tenderness scores of muscle fibers
Loin	Broil	80° C.	35	5.3	9.8	6.0
	Braise	100° C.+	35	3.3	9.3	6.0
	Significance of diff.		n.s.	***	n.s.	n.s.
Bottom round	Broil	80° C.	37	5.3	9.2	5.3
	Braise	100° C.+	39	3.4	6.4	6.5
	Significance of diff.		***[1]	***	***	***

[1]*** Indicates significance at the 0.1% level; n.s., above 5% level.

of variance indicated that these differences were highly significant. Similar differences were reported by Cover and Smith *(7)*. How much of this effect on juiciness was caused by the method of cooking (dry heat versus moist heat) and how much by the difference in the final internal temperature (80° C. versus 100° C.) is not known. In view of the difference in the end point of cooking and the differences in juiciness scores it seems remarkable that the respective weight losses differed by so small an amount (Table 4). In the previous study also, differences in juiciness with these two methods of cooking were obtained and there, also, the weight losses did not differ greatly. If more salivary-stimulating substances were in the browned crust of the broiled steaks than in that of the braised steaks, the subjective impression of juiciness could have been affected thereby. This explanation is not completely satisfactory, however, because the well-done broiled steaks also looked more moist and compact while the well-done braised steaks looked dry and tended to be stringy. Water relationships inside the meat appear to undergo changes which may differ quantitatively under these two conditions of cooking.

When juiciness scores were combined for the 4 conditions of cooking within each cut, the range was very wide. It was wide also for the weight losses during cooking. In general, the juiciest steaks within a cut were those which lost the least weight during cooking. This is shown by the high coefficients of correlation for these two variables within each cut (Table 5).

Tenderness. The shear force values indicated that *longissimus* was most tender (low shear force values) when it was broiled rare and *biceps* was most

TABLE 5

Relationships among weight losses during cooking and the factors of eating quality within the cut

Variables[1]	No. of steaks[2]	Correlation coefficients	
		Loin (*L. dorsi*)	Bottom round (*B. femoris*)
		r	*r*
Juiciness score versus:			
Weight loss	208	−.84***	−.86***
Tenderness score of muscle fibers versus:			
Weight loss	208	−.35***	−.23***
Juiciness score	208	.41***	.24***
Shear	208	−.57***	−.32***
Shear force value versus:			
Weight loss	208	.42***	−.50***
Juiciness score	208	−.38***	.53***

*** — Significant at 0.1% level.

[1] Ranges for steaks were as follows:

	Loin	Bottom round
Weight loss (%)	11–41	10–42
Juiciness score	2.3–9.8	2.3–10.0
Tenderness score	3.3–9.0	3.8–8.8
Shear (*lb.*)	16.0–5.1	14.8–4.4

[2] 26 animals x 4 conditions of cooking x 2 steaks from each animal by each method of cooking = 208 steaks.

tender when it was braised very well-done (Table 3). These findings are of considerable interest because these methods of cooking and these stages of doneness are the traditional ones for cooking loin and round steaks under practical conditions in the home. The heat penetration curves (Figure 1) indicate a flattening of the cooking curves of the well-done broiled steaks between 65° C. and 80° C. Cover *(2, 3* and *4)* has indicated that marked flattening of such curves in roasts is accompanied by greater tenderness of the meat. The well-done broiled steaks from bottom round show this flattening to a greater extent than those from the loin yet the difference in shear force value is small and not significant (Table 3). Heat penetration in steaks occurs much more rapidly than in roasts and does not appear to be associated with tenderization, at least under the broiling conditions of this study.

A "connective tissue score" was reported only when obvious connective tissue was identified by sight or detected during mastication. Because 52 pieces of meat from each method of cooking had been presented to each of four judges, there were 208 opportunities to report connective tissue. For *longissimus dorsi* there were fewer than 20 such reports within any of the 4 conditions of cooking; while for *biceps femoris* there were more than 180 (Table 3). Thus, in only an occasional loin steak did an occasional judge find identifiable connective tissue and even then it was relatively tender. But in bottom round steaks most judges found identifiable connective tissue in most of the steaks and it was tougher than that found in the loin. The different conditions of cooking did not seem to affect greatly either the tenderness of the connective tissue or the frequency of reporting, except when the steaks were braised very well-done (100° C. + 25 minutes). In that case, the connective tissue in both cuts seemed to be somewhat more tender and the number of reports

were somewhat fewer than with the other three conditions of cooking. Braising very well-done employed those cooking conditions usually considered favorable for changing collagen into gelatin and thus tenderizing the connective tissue.

Connective tissue might be expected to be more tender in those cases where less collagen was retained after cooking. Collagen retentions could be obtained only for 6 animals and only for braised loin steaks. Nevertheless, these few determinations indicated that retention of collagen decreased with the longer time of braising. Retentions averaged 64% for loin steaks braised to 85° C. but only 42% for loin steaks braised to 100° C. and held there for 25 minutes. Connective tissue scores indicated greater tendering under the latter condition of braising and obvious connective tissue was found in a fewer number of instances. Thus, the increase in tenderness of the connective tissue seemed to be associated with lower collagen content in braised loin steaks. However, the difference in tenderness of the connective tissue under these two braising conditions is in contrast to the slight difference in scores for muscle fibers or in shear force values (Table 3). Thus, there may be chemical components in meat other than collagen which also affect tenderness. More complete data on collagen content and retention in these two cuts by these methods of cooking would be highly desirable.

A tenderness score for "muscle fibers" was obtained on all samples. When the cut and method of cooking were the same, conclusions based on shears and on the scores of muscle fibers agree: that *longissimus* was significantly more tender broiled rare than very well-done, but showed no significant difference when braised to the two internal temperatures; and that *biceps* was significantly more tender braised very well-done than to 85° C. (Table 3). But, whereas the shears indicated no difference in tenderness of *biceps femoris* broiled to the two stages of doneness (61° C. and 80° C.), the fiber scores indicated that the rare steaks of this cut were significantly more tender. Possible explanations of these variations will be presented later.

When muscles were compared by the same method and the same degree of doneness, both shears and fibers scores indicated that *longissimus* was significantly more tender than *biceps* when broiled rare (61° C.). The traditional explanation for this is the presence of the smaller amount of connective tissue in *longissimus*. But when both cuts were braised very well-done, *biceps* was the more tender. Thus, under certain conditions of cooking, the bottom round which has been regarded as a "less tender" cut, became more tender than the loin which has been regarded as a "tender cut". This contrast in tenderness between the two cuts when cooked very well-done by braising was noted by Cover and Smith *(7)* under the less well-controlled conditions. The marked tenderness of muscle fibers in *biceps* by this method of cooking seemed to be caused partly but not entirely by the conversion of collagen around the fibers into gelatin. Perhaps the muscle proteins were involved also because *longissimus* tended to be stringy and tough while *biceps* tended to be friable or mealy. The cause of this difference in the muscle fibers has not been identified but it is suggested that a method of scoring friableness as well as connective tissue might give useful information.

Wang *(13)* has demonstrated that under certain conditions the sarcolema (the covering of the muscle cell) separates and curls back from the contents

of the muscle cell. If the sarcolema of the muscle fibers of *biceps* but not *longissimus* reacted in this way to braising very well-done, perhaps the contents of the muscle cell would be free to disintegrate readily during chewing or shearing. This possibility cannot be overlooked. But why should the sarcolema of the cells in *biceps* and in *longissimus* differ in this characteristic? If the sarcolema of the cells in both muscles react alike, then the cellular proteins of the two muscles may react differently. Fundamental studies are needed to determine the cause of this difference between the two muscles.

When only well-done steaks were considered, fiber scores and shear force values indicated that there were only small insignificant differences in tenderness between loin steaks cooked well-done by broiling versus braising; but that bottom round steaks were significantly more tender braised well-done than broiled well-done (Table 4). Cover and Smith *(7)* reported similar findings for ¾-inch steaks. Thus, these data on 1-inch steaks cooked under the better controlled conditions confirm similar findings from the previous study.

Inter-relationships among factors of eating quality. That the judges may have allowed juiciness to influence their tenderness judgments of muscle fibers could be inferred from the following observations: in these rare broiled steaks significant positive correlations were obtained between fiber score and juiciness score, (.570*** and .373** for loin and bottom round, respectively); but those between shear force value and juiciness score were not significant (−.114 and .102 for loin and bottom round, respectively). Perhaps the juiciness of the rare broiled steaks induced swallowing before the essential toughness or tenderness was assessed.

On the other hand, these observations may be interpreted in another way. One aspect of tenderness is the ease with which the teeth sink into the meat. This may be called "softness". Softness is often noted in rare broiled steaks which also are the juiciest ones. Consequently it may have been the softness of the steak rather than its juiciness *per se* which influenced the fiber scores of the rare steaks in this study. Moreover, softness might tend to mask a judge's estimate of other tenderness-toughness components which are more important in determining shear force value. It is suggested that a method of scoring softness might give useful information.

Other correlation coefficients were calculated by using as one series all of the steaks from one cut, without distinguishing the method of cooking. Under these circumstances, fiber score in both cuts was significantly associated negatively with weight loss and positively with juiciness score (Table 5). The lower shear values (more tender meat) also tended to be associated with lower weight losses and higher juiciness scores but only in loin steaks. In bottom round steaks, lower shear values tended to be associated with higher weight losses and lower juiciness scores. These observations emphasize the differences between the two cuts in some of their responses to cooking.

Coefficients of correlation between shear value and fiber score were highly significant (Table 5) but were considerably lower than the 0.9 reported by Ramsbottom and Strandine *(11)* and the values −.73 to −.89 by Cover and Smith *(7)*. Paul, Bean and Bratzler *(10)* reported coefficients of correlation between shear force value and tenderness score of −.369 and −.404 (both

significant at the 1% level). In the earlier work the tenderness scores were a composite of all subjective impressions of tenderness or toughness. In the present study, however, separation of the subjective impressions into those coming from muscle fibers and those from obvious connective tissue was attempted. No such distinction is made by the shearing device and perhaps as high correlation as was found previously ought not to be expected from the present data. Then, too, in the earlier work in this laboratory, none of the meat was cooked rare and differences in softness were not marked. Of the 8 steaks presented at each judging session in the present study, two were broiled rare and two were braised well-done, thereby providing a wide range in juiciness and softness. The judges may have become confused in trying to combine softness and other tenderness components into one score. Perhaps if tenderness were divided into several components it might be easier to note associations with certain cooking conditions and with other measures of eating quality. This is proposed for a subsequent study with other animals.

SUMMARY AND CONCLUSIONS

The steaks for this study were taken from two muscles from each of 26 yearling steers and were cooked to two degrees of doneness by broiling (internal temperatures 61° C. and 80° C.) and by braising (internal temperatures 85° C. and 100° C. plus a holding period of 25 minutes). The meat was tested for tenderness with a mechanical shearing device and a panel of judges. Scores for tenderness of obvious connective tissue and scores for tenderness of muscle fibers were obtained instead of the usual composite score for tenderness. Juiciness scores were obtained also.

Juiciness scores decreased with increasing doneness within each method of cooking. The well-done broiled steaks had higher juiciness scores than the well-done braised steaks of each cut and also looked more moist and compact. Yet weight losses were very similar for these two conditions of cooking. This may indicate that the water combinations inside the meat after broiling to 80° C. differ from those present after braising to 100° C. and being held there for 25 minutes.

Shear force values indicated that *longissimus dorsi* was most tender broiled rare (61° C.) but that *biceps femoris* was most tender braised well-done (100° C. and held there for 25 minutes). Thus, severe cooking conditions appeared to toughen the loin steaks but the most severe of the four cooking conditions seemed to tenderize the bottom round steaks.

Score for muscle fibers was significantly correlated with shear force value. The coefficients were −.57 for *longissimus dorsi* and −.32 for *biceps femoris*. Each was highly significant.

Obvious connective tissue was found less frequently and was more tender in *longissimus* than in *biceps*. The connective tissue appeared to be tenderized to the greatest extent by the most severe of these four methods of cooking, braising to 100° C. and holding there for 25 minutes.

Evidence is presented that tenderness in meat has more than one component and that *longissimus dorsi* and *biceps femoris* muscles differ markedly in their tenderness response to certain cooking conditions.

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AFTER-COOKING DISCOLORATION OF POTATOES: POSSIBLE INVOLVEMENT OF POLYPHENOLIC CONSTITUENTS

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After-cooking discoloration of potatoes has been a serious problem in several growing areas east of the Mississippi River for more than 20 years. This problem was acute with the 1953 crop but occurred in only rare lots of the 1954 crop. Several hundred samples of potatoes from the 1954 Maine crop were screened by staff members of the Maine Agricultural Experiment Station but little incidence of this type of discoloration was found. Blackening of cooked potatoes was again troublesome with the 1955 crop.

The extensive literature on after-cooking discoloration of potatoes was recently reviewed by E. Yanovsky *(22)*, and will not be considered in detail here. This type of discoloration usually occurs as greying or blackening, in greatest intensity at the stem end just beneath the skin. Flesh of affected tubers typically appears normal in the raw state.

After-cooking discoloration is not only encountered in home and restaurant cooked products but in all types of processed potato products. This discoloration can cause difficulties to processors, particularly in canned potatoes, frozen products, and dehydrated potato products. Caldwell *et al. (3)* reviewed thoroughly the various types of discoloration encountered in the processing and use of dehydrated potatoes.

Water blanching and acid dipping treatments of the raw, peeled stock are of limited value in inhibiting after-cooking discoloration. Sulfiting is of some help but cannot be used with potatoes to be processed with meat, since foodstuffs containing meat must be sulfite-free for shipment in interstate commerce.

In the past 10 years, at least two investigations have been made of a colorless, fluorescent substance extracted from potatoes with acidified alcohol. Lewis and Doty *(12)* believed this substance to be the precursor of the pigment causing potato blackening, since it was readily converted to a black pigment by heating above 35° C. However, Bowman and Hanning *(2)* concluded that there was no clear relationship between the extent of fluorescence obtained with this substance and the tendency for potatoes to blacken.

Many possible causes have been suggested for after-cooking discoloration, but few facts have been established and much of the published data are contradictory when compared. Some of the factors proposed as being related to after-cooking discoloration are: potato variety; soil pH and composition; growing conditions; storage conditions; ratio of protein nitrogen to nonprotein nitrogen; and the amounts of tyrosinase, iron, phosphorus, and manganese in the tubers.

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If the reaction or reactions responsible for pigment formation can eventually be defined, then discoloration can possibly be prevented by use of certain methods in growing potatoes or by some permissible treatment during processing. One popular theory is that oxidation of polyphenolic compounds, perhaps catalyzed by the presence of iron, forms the pigment of after-cooking discoloration. Smith, Nash, and Dittman *(17)* stated that *o*-diphenolic compounds upon oxidation may produce the pigment that darkens potatoes. Juul *(8)* believes that bivalent iron in potato juice combines with *o*-diphenol to give a light-colored substance. Upon exposure to air, oxidation leads to formation of a dark-colored ferric compound.

Juul *(8)* found that cooking potatoes in a dilute solution of sodium hydrogen pyrophosphate prevented after-cooking discoloration, presumably because of sequestration or chelation of the iron. Smith and co-workers have obtained some success in the use of sequestering and chelating agents to prevent discoloration by applying foliar sprays during growth of the plants *(18)* and by dipping peeled potatoes that are to be kept at least 24 hours before cooking *(6)*. Cheng and Hanning *(4)* recently assayed various lots of potatoes for total tannin content, in which study they found chlorogenic acid to be the only polyphenol present in detectable amounts in the flesh. Caffeic acid was found in the skin extract in addition to chlorogenic acid.

Kiermeier and associates *(9, 10, 11)* have quite recently studied chemical and enzymic changes as possible causes for the discoloration of steamed potatoes. Their data indicated that only a small amount of chlorogenic acid is present in the German potato variety "Ackersegen" in raw tissue but that about 10 times as much is present in steamed potatoes. The findings of these German workers suggest that chlorogenic acid is hard to release physically from raw tissue or is bound in some sort of chemical complex from which it is released during cooking. However, the results that we have on comparison of the chlorogenic acid contents of raw and cooked tissue do not agree with this.

Kiermeier and co-workers *(9, 10, 11)* found quinic acid present in potatoes but felt that it did not contribute to discoloration. Caffeic acid was present in too low a concentration for them to determine it quantitatively. They believe that bivalent iron, chlorogenic acid and caffeic acid, and oxygen are essential for pigment formation.

Having at our disposal a series of lots of potatoes obtained from different parts of the country and exhibiting varying degrees of blackening after cooking, there were 3 principal objectives in the work reported here:

1. To isolate as many polyphenolic constituents as possible from extracts of the tissue.
2. To search for differences in the number and relative amounts of various polyphenols in blackening tissue as compared with non-blackening or normal tissue.
3. To determine quantitatively the chlorogenic acid contents of potatoes of different blackening tendencies and in various sections of individual tubers.

All lots of blackening potatoes were selected after discoloration had been noted during the winter storage period. No data are presented on possible

changes in the polyphenol content during maturation of the tubers and storage of the mature potatoes.

We have used two-dimensional paper chromatography both to separate the polyphenolic constituents and to obtain data on the amounts of chlorogenic acid present in extracts.

EXPERIMENTAL

Preparation of concentrated extracts. Twelve samples of potatoes representing various degrees of blackening were examined to determine the number of polyphenols present. They were then assayed for chlorogenic acid. Samples of each lot of potatoes were taken from the stem end, bud end, skin, and whole potato. The stem and bud end samples were obtained by taking a ½-inch long by ⅞-inch diameter plug out of each end of the potato with a No. 15 cork borer. The skin samples were prepared by removing the skin with a potato peeling knife. About ⅛-inch of flesh adheres to the skin. The whole potato samples were prepared by taking a representative sample of cooked mash (including skin).

The preponderance of our data was taken on cooked potato samples. However, with 3 lots of blackening potatoes and one control lot, corresponding samples were taken of both cooked and raw tissue for chlorogenic acid assays.

Two methods of sample preparation were used. The first (Method A) is more time consuming, but results in a dry final product for assay. However, the second (Method B) required no drying step, and the final volume of purified concentrate more accurately represented the extract from a given weight of potatoes.

Method A. Method A was derived from that described by Johnson and Schaal *(7)*, with modifications to make it quantitative. One hundred grams of the potato fraction to be assayed (stem end, bud end, skin, or whole potato) was steamed for 30 minutes and cooled. It was then ground in a Waring blender [e] for 5 minutes with 300 ml. of hot 95% ethanol. The material was diluted to 2 liters with 70% ethanol. After standing overnight, the mixture was filtered and 1800 ml. of the filtrate was concentrated to about 15 ml. in a rotating evaporator below 40° C. The concentrate was extracted in a separatory funnel with 300 ml. of hydrocarbon solvent (Skellysolve B [e]) to remove waxy and fatty material. The organic solvent in the aqueous layer was boiled off on a steam bath to prevent splashing during subsequent oven drying. A yellow or brown material, which was not removed, floated on top of the aqueous solution after removal of the organic solvent. The solution was then dried in a vacuum oven at slowly elevated temperatures not exceeding 40° C. for 5 hours. When almost dry, the material puffed up to give a friable texture that permitted drying to a moisture-free basis. Although quite hygroscopic, the dried substance could be weighed quickly without any difficulty. In early experiments the dried material was re-extracted with organic solvent, but this was later found to be unnecessary. The powder was stored in a desiccator because of its hygroscopicity. To prepare a batch of the final solution, 300 mg. of the powder was dissolved in 2 ml. of 30% isopropanol and this was allowed to stand in a refrigerator for the settling out of any sediment. Sample aliquots were then either filtered off or removed without shaking the solution. The sediment contained a considerable amount of asparagine in addition to fatty material.

Method B. Up to the point of drying the solution in a vacuum oven, *Method B* was the same as Method A, except that the material was concentrated down to at least about 8 ml. in the rotating evaporator. After expelling the Skellysolve on the steam bath, the material was not oven-dried, but was poured from its dish into a 25-ml. volumetric flask and the dish was washed with such amounts of water and isopropanol that the washings gave a final solution of about 30% isopropanol. The solution, at first cloudy because of emulsified droplets, was placed in a refrigerator until clear, then filtered or used without shaking.

Method A was developed first, and was used to prepare all the samples except one (57–3, Table 1), but Method B is preferred unless a stable, dried extract is desired for other assays.

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[e] Mention of trade names does not imply that products are recommended or endorsed by the United States Department of Agriculture over others not mentioned.

Paper chromatographic methods. The most satisfactory solvents tried were those used by Cartwright, Roberts, Flood and Williams *(3a)* in their study of tea. We used their solvents in the reverse order, i.e., 2% acetic acid in the first direction and butanol, acetic acid, water (4:1:2.2) in the second which gave better results with potato extracts. The descending technique was employed, using a Chromatocab ° and Whatman No. 1 ° paper, 46 by 57 cm. Large sheets of filter paper were used because substances having high R_f values in the first solvent were thereby separated sufficiently from the polyphenols so as not to interfere with the development in the butanol-acid system. Two methods of extract preparation were used, as described below. A spot of concentrated extract about 2 cm. in diameter was applied at the starting line and developed in the long direction first. About 7 hours was taken for development in acetic acid as solvent and about 17 hours (overnight) for the second solvent system.

The completed chromatogram was observed under ultraviolet light. Several spots fluoresced blue-white. After exposure of the paper to NH_3 vapor, other spots appeared under ultraviolet light. The paper was then sprayed with Benedict's solution, which was used by Gage, Douglass, and Wender *(5)* for flavonoid-type compounds. We found this solution useful for color differentiation of certain types of polyphenols, as outlined in the Results and Discussion section.

Some of the spots obtained on the chromatogram were very faint. In order to concentrate further the polyphenols before regular two-dimensional chromatography, the following scheme was used. An extract of potato peel was applied as a streak across the starting line and the streak developed with 2% acetic acid. Four UV-fluorescent bands were obtained, the fastest running one being discarded since it contained most of the inorganic salts and amino acids. The other 3 bands were eluted separately, the eluates concentrated, applied to large sheets of paper, and chromatograms developed using the two solvent systems mentioned above. The papers were then sprayed with Benedict's solution. The procedure was used for chromatograms representing the fluorescent polyphenols found in potatoes. Tyrosine was not included in consideration of the polyphenols since it is non-fluorescent.

Chlorogenic acid assay. The concentrated extracts prepared by either of the two methods were applied to large sheets of Whatman No. 1 filter paper in suitable amounts, about 30 or 40 mcl. for samples extracted from skin and 60 to 80 for the others. As was the case with formation of the complete chromatogram, about 7 hours was required for development with the first solvent and about 17 hours for the second solvent system. Sufficient equilibration time was allowed with each solvent. The papers were not sprayed in the quantitative determination. The main chlorogenic acid spot of the chromatogram was located under ultraviolet light and circled. It is assumed that the quantity of the principal isomer of chlorogenic acid bears a definite relation to the amounts of other isomers present or formed in the experimental procedures and that it is valid to compare relative amounts of chlorogenic acid in different samples of potatoes on this basis. The area of paper comprising the spot was cut into pieces and eluted with 6 ml. of 50% *n*-propanol in a stoppered tube. The color was developed with 2 ml. of a solution containing 1% $NaNO_2$ in 1% acetic acid. This reagent was used in 10% acetic acid by Paech and Ruckenbrod *(14)*. The stoppered tubes containing the paper were heated at 60° C. for ½ hour, cooled, and the liquid transferred to calibrated tubes. These were read in a spectrophotometer at 445 mμ. The readings were compared with a standard curve prepared according to Ayres *(1)*. The most accurate reading range is between 40 and 300 mcg. of chlorogenic acid. This is, of course, limited by the amount of potato extract which can be applied to the paper without resulting in streaking during subsequent chromatography. Most situations permitted application of 50 to 100 mcg. of chlorogenic acid. Occasionally, however, only about 30 mcg. of chlorogenic acid was obtained from as much as 80 mcl. of potato extract.

The chlorogenic acid sample used for the standard was dried in a vacuum oven at 70° C. before use. It was found to be a hemihydrate, as reported by Moores, McDermott, and Wood *(13)*, after drying to constant weight over P_2O_5 at 135° C. The values in Table 1 are reported as anhydrous chlorogenic acid.

Sample calculation for chlorogenic acid assay of extract prepared by Method A. Upon extracting 100 g. of potato tissue in 2000 ml. of 70% alcohol, 1800 ml. of the filtered extract was taken for concentrating and preparation of the dry powder. Let us

TABLE 1

Chlorogenic acid contents of potatoes of different varieties, sources, and blackening tendencies

Sample	Blackening extent	% Solids	Variety, source	Chlorogenic acid, mg./100 g. fresh tissue			
				Stem	Bud	Skin	Whole
N-2	None	20.5	White Rose, Calif., 1956	12	32	30	8
57-3	V. slight	18.6	Katahdin, Pa., 1956	7	10	42	11
5	Slight	20.0	Rus. Burbank, Mich., 1955	13	23	57	11
4	Slight	21.9	White Rural, Mich., 1955	14	38	95	18
9	Mod.	18.5	Katahdin, Maine, 1955	13	19	42	13
1	Mod.	23.0	Unnamed,[1] N.D., 1955	24	37	167	32
2	Mod.	20.2	Kennebec, Maine, 1955	23	39	50	16
7	Mod. severe	19.6	Var. unknown,[2] Maine, 1955	13	17	35	6
8	Severe	23.2	Katahdin, Pa., 1955	18	26	....	....
3	Severe	21.2	Sebago, Maine, 1955	13	12	32	9
57-1	Severe	22.4	Kennebec, Maine, 1956	13	20	....	....

[1] Obtained from W. G. Hoyman, North Dakota Agr. Expt. Sta., Fargo, N. D.
[2] Obtained through Maine Agr. Expt. Sta., Orono, Maine.

say that 2.74 g. of dry powder was recovered from the 70% alcohol extract after extraction with Skellysolve B. From this dry powder, 0.300 g. was weighed out and dissolved in 2 ml. of 30% isopropanol. In 50 mcl. pipetted from this concentrated extract 48 mcg. of chlorogenic acid was found. Then, the quantity of chlorogenic acid present in 100 g. of the potato tissue is computed in the following manner:

$$\frac{2000 \times 2.74 \times 2 \times 48 \times 10^{-6}}{1800 \times 0.300 \times 0.050} = 0.02 \text{ g. chlorogenic acid}$$

Data in the tables are given in terms of milligrams of chlorogenic acid per 100 g. of potato tissue.

This calculation disregards the volume occupied by the insoluble material in the 2-liter flask in which the alcoholic extraction was made.

Determination of total polyphenols. Total polyphenolic constituents were determined by the method of Rosenblatt and Peluso *(16)*, using standard chlorogenic acid as the basis for expressing total polyphenols. The same powdered extracts were used for the total polyphenols determinations as were employed for the determination of chlorogenic acid by paper chromatography. Spectrophotometric absorption measurements on these colored solutions were made at 650 mμ.

RESULTS AND DISCUSSION

Polyphenolic substances considered individually. Figure 1 represents diagrammatically a chromatogram of the polyphenolic compounds typically extracted from potato peel by 70% alcohol. Peel extracts were used for observation because the polyphenols are most abundantly present in the skin and the flesh immediately beneath. Six of the spots that occur most commonly in chromatographing the extracts are denoted by underlining the numbers.

In semiquantitative work, the polyphenolic content of potato skin was compared with that of the flesh immediately underneath the skin. The skin of cooked potato was carefully separated manually from the flesh of the layer removed in paring with a potato peeling knife. Chromatographs of the purified, concentrated extracts of these two fractions showed that on the dry basis the concentration of polyphenols in the skin was about twice that in the fleshy tissue.

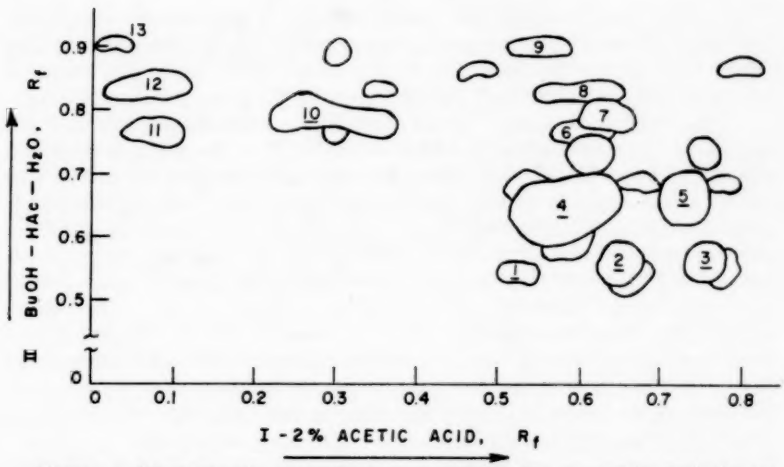

Figure 1. Diagrammatic chromatogram of polyphenolic compounds typically extracted from potato peel by 70% alcohol.

The polyphenolic substances were first separated into three fluorescent bands on paper as indicated under "Paper Chromatographic Methods." These bands, after elution and concentration, were resolved separately by two-dimensional paper chromatography. Figure 1 is a composite schematic representation of these individual chromatograms. The first band (that nearest the acetic acid starting line) yielded, after eluting, concentrating, and chromatographing, the group of spots numbered 11, 12 and 13. These appeared in the position expected of substances concentrated in the first band. Simultaneously, a second group of spots numbered 6, 8 and 9 appeared at a position well beyond that of the first group, as shown in Figure 1. It is believed that 6, 8 and 9 represent isomers of 11, 12 and 13. Williams *(20)* has expressed the belief that cis- and trans-isomers of cinnamic acid derivatives exist in equilibrium. Following separation of the cinnamyl isomers by chromatography with 2% acetic acid as solvent, Williams found that a second chromatographic treatment brought about further resolution in which each form yielded a portion of the other isomer. In addition to chlorogenic and caffeic acids, the other polyphenols of the potato may well be of the cinnamyl type, since many of these compounds are known to be present in plants *(19, 20)*.

The second band contained what appeared to be caffeic acid (spot number 10) in the expected position, and three fluorescing spots. There also appeared in the chromatography of the extract from the second band a spot (number 7) believed to be an isomer of caffeic acid. Tentative identification of the caffeic acid isomers is based on the R_f values of Williams *(20)* and the color reactions. Benedict's solution can be used to differentiate caffeic acid, chlorogenic acid, and their isomers from the other polyphenolic compounds. Spots formed by the caffeic and chlorogenic acids are yellow in visible light and dark under ultraviolet light, while the other spots do not appear in ordinary sunlight but are blue under ultraviolet.

The third band contained the largest number of polyphenolic compounds of the three bands. Upon chromatographing this band, following elution and concentration of the constituents as done with the other bands, four forms of chlorogenic acid were obtained. Spot number 4 is the principal form of chlorogenic acid and 5 is its isomer. Spot number 2 is neo-chlorogenic acid and 3 is its isomer. These 4 constituents were identified by R_f values given in the literature *(15)*. A number of blue-fluorescing spots also appeared as well as spot number 1, which may be another form of chlorogenic acid, since it exhibited the same color reactions as the chlorogenic acid group. Spots representing chlorogenic acid isomers were identified by the following color reaction: the paper was exposed to NH_3 vapor and examined under ultraviolet light for spots colored "duck egg" green (greenish yellow), which is characteristic of the chlorogenic acids *(3)*. Spots having R_f values below 0.50, in the butanol-acid system, are not shown in Figure 1 because they are either vary faint or not clearly defined. Except for the chlorogenic and caffeic acids, we have not identified up to the present time the other compounds whose spots appear in the chromatogram.

Quinic acid was not given attention in this paper since it is not a phenolic compound. However, its presence in the aqueous alcoholic extracts of potatoes was detected on paper chromatograms, as evidenced by a specific color test *(3)*.

Table 1 gives values of chlorogenic acid found in whole, cooked potato and in various fractions. Confirming the results of others, chlorogenic acid content was found to be always higher in the skin than in the other parts of the potato. This was no more pronounced in potatoes that blacken severely than in those that do not. In any given section of potato, wide variation in chlorogenic acid content was found from lot to lot. Counter to blackening tendency, chlorogenic acid content was greater in the bud than in the stem end. However, this was not true with sample number 3, one that exhibited severe blackening.

Table 2 presents comparable data on the chlorogenic acid contents of bud and stem end sections of raw and cooked potatoes. Determinations to be compared were made on potatoes of a given lot and on the same day. The values obtained before and after cooking were nearly the same.

TABLE 2

Comparison of chlorogenic acid contents of raw and cooked potato tissue

Sample	Blackening extent	Section	Chlorogenic acid expressed as mg./100 g. of fresh potato	
			Raw	Cooked
N-1	None	Bud	18	16
		Stem	9	8
7	Moderate-severe	Bud	20	17
		Stem	10	13
9	Moderate	Bud	19	19
		Stem	12	13

TABLE 3

Precision of the method of determining chlorogenic acid in high and low ranges

Number of replicates	Mg. chlorogenic acid/100 g. fresh potato		
	Range	Mean	Standard deviations[1]
10	164–182	172.7	5.3
10	34– 38	36.6	1.6

[1] Standard deviation $= \sqrt{\frac{\Sigma \text{ squares of deviations}}{\text{number of replicates} - 1}}$

The precision in the estimation of chlorogenic acid by the paper chromatographic method is indicated in Table 3.

Standard amounts of chlorogenic acid were added to extracts of potato before the concentrating step in order to check percentage recovery. The recovery of added chlorogenic acid ranged from 88–98%. This indicates a fairly high degree of accuracy in determining the amount of chlorogenic acid present.

Polyphenolic constituents considered as a group. In addition to the chlorogenic acid determinations, analyses were made for the total polyphenolic content of extracts of the bud, stem, skin, and whole potato tissue from the same series of potatoes with varying blackening tendencies. Table 4 shows the data obtained on the stem-end sections. These data, as well as those for every sample from the other sections of the tuber and from the whole potato, show that the total polyphenolic content of potatoes, in addition to their constituent chlorogenic acid content, is appreciable. Comparison of Table 4 with Table 1 shows that the ratios of total phenols to chlorogenic acid in the stem ends of the tubers ranged from 5 to 12. In the skins the total polyphenolic content ranged from 2 to 6 times that of the chlorogenic acid content.

In Table 4 no correlation is apparent between total polyphenolic content

TABLE 4

Total polyphenolic contents of stem-end sections of cooked potatoes

Sample	Blackening extent	Total polyphenolics[1] expressed as mg./100 g. of:	
		Fresh tissue	Dry tissue
N-2	None	122	595
57-3	V. slight	87	468
5	Slight	104	520
4	Slight	170	777
9	Moderate	138	745
1	Moderate	107	464
2	Moderate	109	540
7	Moderate-severe	134	683
8	Severe	146	628
3	Severe	92	432
57-1	Severe	92	411

[1] Determined by Rosenblatt and Peluso method, with chlorogenic acid as standard of reference.

and blackening tendency. As a rule, the stem ends of the potatoes, where blackening is most acute, were not found to contain any more polyphenols than the bud end.

SUMMARY AND CONCLUSIONS

Ten lots of potatoes exhibiting after-cooking discoloration were examined in comparison with normal, non-blackening potatoes regarding the possible relationship between the amount of polyphenolic compounds present and the extent of blackening. In two-dimensional chromatography of concentrates prepared from 70% alcohol extracts of potato tissue, about 27 spots were obtained representing substances having certain properties of polyphenols. In agreement with the results of others, the polyphenols were found to be concentrated in the skin and immediately-underlying tissue. However, counter to blackening tendency, the polyyphenols were present in no greater amount in the stem end than in the bud end of the tuber. In general, potatoes from blackening lots did not have a greater variety of polyphenols present or a higher concentration of chlorogenic acid than those from nonblackening lots. Chlorogenic acid assays were made on tissue from various sections of the potato, using a paper chromatographic method. Chlorogenic acid is believed to be the principal polyphenol in potatoes, but determinations of total polyphenols by the method of Rosenblatt and Peluso showed that an appreciable fraction of the polyphenols consists of compounds other than chlorogenic acid. Although polyphenols may be involved in the after-cooking discoloration of potatoes, the tendency toward discoloration apparently is not proportional to the concentrations of chlorogenic acid and other polyphenols present.

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STUDIES OF PROCESSED ALL-GREEN ASPARAGUS. I. QUANTITATIVE ANALYSIS OF SOLUBLE COMPOUNDS WITH RESPECT TO STRAIN AND HARVEST VARIABLES, AND THEIR DISTRIBUTION WITHIN THE ASPARAGUS SPEAR[a]

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In 1935, Culpepper and Moon *(2)* reported their studies on the composition of the developing asparagus shoot. Chief amongst the items studied were the changes in solids content, sugars, titratable acidity, total astringency and nitrogen content. Numerous other studies have been conducted on the ascorbic acid content of asparagus before and after processing *(7, 11, 14)*. Thornton *(13)* reported that the vitamin C content in asparagus shoots increased from 15 to 150 mg. per 100 g. of fresh weight as the spears matured from the dormant stage underground to 100 cm. in height above ground. Wolf *(15)* found that green asparagus was higher in vitamin C than white and that the concentration was greatest in the tip and decreased along the stem with the cortex being richer in vitamin C than the interior.

Campbell *(1)*, in 1939, reported that the yellow precipitate, which occurs when all green asparagus is processed in glass, is a flavonone or flavonol glucoside. By spectral analysis in 1948, De Eds *et al., (5)* identified the yellow crystalline precipitate as rutin. Later, Stevenson *(12)* studied the rutin content of asparagus and found it to be highest in the tip of the green asparagus spear. As the asparagus matured the concentration of rutin increased.

It is natural to assume that changes in the composition of fresh asparagus would be reflected in the processed product. All-green asparagus processed as described later was therefore used to determine quantitatively the influence of the height of asparagus at harvest, the diameter of the spear at harvest and the section of the spear on the concentration of such soluble components as titratable acidity, rutin, vitamin C and Folin-Denis reacting substances.

EXPERIMENTAL

A. Sample preparation

All asparagus, unless otherwise specified, was harvested on the Davis Campus from Yolo fine sandy loam to silt loam prior to 8:30 a.m. and transported less than one mile to the experimental processing plant. The asparagus was selectively harvested at 3 heights above ground, 3–6 inches, 6–12 inches, and 12–18 inches.[b] Upon receipt at the plant, the asparagus was spray washed with water (60 p.s.i.g. at the jets) in the washing section of a Food Machinery and Chemical Corporation cup-down lye peeler. Following washing, each lot was reduced to a 4-inch tip section by trimming and further subdivided

[a] Presented at the Seventeenth Annual Meeting of the Institute of Food Technologists, Pittsburgh, Pennsylvania, May 15, 1957.

[b] In the remainder of this report these heights will be referred to as short, medium, and tall, respectively.

into 3 spear diameters (measured 4 inches below tip): less than 3/8 inch, 3/8 to 5/8 inch and greater than 5/8 inch.[c]

All lots were cut into one-inch sections, thoroughly mixed and weighed portions taken as samples. These were placed in either 303 or 8-ounce (glass) jars and a measured amount of distilled water was added. Glass was used to prevent the tin contamination of the asparagus, which would interfere with the rutin analysis. Stainless steel knives and trays and lacquered lids were used to prevent metal contamination of the product. The spears were not blanched nor were the jars exhausted. They were steam-jet closed, processed at 248° F. for 16 minutes following a 5-minute come-up period. Ten p.s.i.g. over-riding air pressure was used to prevent loss of lids and 25 p.s.i.g. air pressure during cooling to 150° F. Upon removal from the retort, the jars were shaken to macerate the tissue and were allowed to cool to room temperature. Slightly less than 3 hours elapsed between harvest and the destruction of the living tissue by heat.

1. Height and diameter study. Five of the strains of asparagus available on the University of California Davis campus were used in this study.[d]. Although they were harvested at different dates, each strain was treated in an identical manner. Each strain was harvested at 3 heights above ground, and after being washed, the 4-inch tip section was removed. These 4-inch tip sections were then subdivided into the 3 size grades, resulting in a total of 9 lots for each strain. Further preparation and processing was conducted as described previously. Samples consisted of five 100 g. replicates processed in 303 glass jars in 350 ml. of distilled water.

2. Spear section study. For this study, Strain 711 was harvested at the 3 heights above ground described previously. Cut spears were washed and only those of medium diameter and with 4 inches or more of green were studied. The spears were cut into 2-inch sections from the tip downward; the short spears were cut into three 2-inch sections and the medium and tall spears into 4 sections. The number of spears and the total weight of each section within each height were recorded. Samples of 50 g. were packed in 8-ounce glass jars with 150 ml. of distilled water and processed as described.

3. Seasonal change. Two strains of asparagus, 500 and Mary Washington, were harvested at weekly intervals for 3 weeks from randomized test plots. Seventy-five gram portions of the 4-inch tip cuts of the medium diameter medium height spears were processed in 125 ml. of distilled water in 8-ounce jars for this study.

B. Analytical procedures

Forty-eight hours prior to analysis, each sample was heated in a steam chamber at 190° F. for 15 minutes, shaken while hot, and allowed to cool in air to further complete maceration of tissues. It was then analyzed for pH, titratable acidity, ascorbic acid, rutin and Folin-Denis reacting substances by the procedures described below.

It is assumed that a chemical equilibrium of the contents of the jar exists at this stage, and that 1 ml. of filtrate prepared as described later equals 1 g. of total contents. Results are computed to an original fresh weight basis on this assumption. The values reported are the average of 5 replicate analyses.

1. pH. pH was determined by a Beckman Model H2 pH meter on an aliquot of the thoroughly mixed contents of the container. The remaining contents of each jar were filtered by suction using Hy-flo Super-cel as a filter-aid. A minute quantity of Dow Corning A. F. emulsion was added to the sample prior to filtration to prevent foaming. The first 25–50 ml. of filtrate and pulp was discarded. Aliquot portions of the clear filtrate were used for all subsequent analysis.

2. Ascorbic acid. The ascorbic acid content of the filtrate was determined by the National Canners Association photometer method *(9)* using metaphosphoric acid to acidify the samples. Results were computed to mgms. per 100 g. of fresh weight.

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[c] These will now be referred to as small, medium and large respectively. Since a spear may be oval in shape, the largest diameter was used to define spear size.

[d] Propagation from selections made by Mr. G. C. Hanna, Department of Vegetable Crops, for *fusarium* wilt resistance as part of a project devoted to studies on re-planting land formerly used for this crop.

3. Titratable acidity. This determination was conducted by titrating a filtered sample with .05 NaOH to a pH of 8 using a Beckman automatic titrimeter. The results are expressed as milliequivalents per 100 g. of fresh weight.

4. Folin-Denis. The Folin-Denis reacting substances were determined by a modification of the AOAC procedure *(10)*. An aliquot of the clear filtrate was diluted to give a Klett-Summerson photometer reading of below 200 with a No. 66 filter. One ml. of this solution was added to a 50 ml. volumetric flask containing 20 ml. of distilled water. Then 3 ml. of Folin-Denis reagent and 10 ml. of saturated sodium carbonate were added, the mixture diluted to volume and thoroughly shaken. A blank using distilled water was similarly prepared. After these samples were held in the dark for one hour, the optical density was measured in a Klett-Summerson colorimeter against the blank using a No. 66 red filter (at times it was necessary to filter the blank to remove a white precipitate). These readings were converted to their rutin equivalent by comparison with a standard curve plotted for rutin. The rutin (Nutritional Biochemical Corporation) was dried at 60° C. under vacuum for 24 hours before use as a standard. A standard curve was similarly prepared for the reaction of Folin-Denis reagent with ascorbic acid. This permitted the conversion of ascorbic acid content as determined by the National Canners Association photometer method to its rutin equivalent as measured by Folin-Denis. A factor of 1.65 was established for this conversion.

5. Rutin. Rutin was determined by a procedure shown to yield satisfactory results by preliminary investigations. The work leading to its adoption is discussed further on in this paper.

A 2 M pH 5.4 acetate buffer was mixed with an equal volume of 0.1 M $AlCl_3$ solution to yield a mixture with a pH of 5.0. The acetate buffer was adjusted to pH 5.4 prior to adding the $AlCl_3$ solution as attempting to adjust the mixture to exactly pH 5.0 with strong base after addition of the $AlCl_3$ caused the formation of an alumina hydroxide cloud. It was critical that the mixture have a final pH of 5.0.

Two ml. aliquots of the solutions diluted for Folin-Denis determination were placed in separate test tubes. Four ml. of 1 M pH 5.0 buffer was added to one tube to serve as a blank and 4 ml. of the pH 5.0 acetate buffer—$AlCl_3$ mixture was added to the other to produce a color complex. Each tube was shaken and after 15 minutes the absorbance of the colored complex was determined against the blank in a Beckman Model DR automatic Spectrophotometer over a range of 410–420 mμ. The peak absorbance at 416 mμ was compared with a standard curve plotted for rutin. It was found desirable to keep the absorbance readings between 0.2 and 0.8.

RESULTS AND DISCUSSION

Several methods for the qualitative or quantitative analysis of rutin by the formation of a color complex with aluminum have been published *(4, 6, 8)*. Some of these methods were devised for the determination of the purity of rutin-tablets. Others require tedious extraction or purification procedures. A method was developed for the direct quantitative analysis of rutin in boiling water extracts of asparagus.

It was first necessary to determine the specificity of the aluminum chloride reaction for rutin in water extracts of asparagus. Various concentrations of extracts were separately chromatographed on Whatman No. 1 filter paper in two solvents: n Butanol-acetic acid-water 4:1:5 v/v and phenol-water 75:25 v/v. The chromatograms were then developed by spraying with a 0.1 M aqueous aluminum chloride solution followed by 1. M aqueous potassium acetate solution. Brilliant yellow spots developed having R_f values corresponding to those of known rutin. A second but almost undiscernible series of spots developed which was due to the reaction between anthocyanins and aluminum chloride. Thus, the color complex developed in water extracts of asparagus is due almost entirely to rutin. Further support of this was obtained by the spectral analysis of the water extracts before and after the addition of

a buffered aluminum chloride solution. Absorption spectra of aqueous extracts in the range of 500 to 325 mμ were identical to those of pure rutin. Thus direct rutin analysis of a boiling water extract of asparagus by the formation of a color complex with aluminum is possible.

In the development of this method it was observed that maximum sensitivity under the above conditions was obtained at approximately pH 4.0. At pH 3.0 there was little color formation and above pH 4.0 the sensitivity of the method decreased. The decrease at higher pH values was due to a shift in the absorption spectra of the blanks. At pH 4.0 pure rutin solutions are colorless, at pH 6 slightly yellow and at pH 7 distinctly yellow. Thus, at the high pH's the sensitivity was decreased due to the increased optical density of the blank. Clouding of samples also occurred at the higher pH values and occasionally occurred at pH 6.0. Acidification of the asparagus filtrate to pH 4.0 also caused clouding. Filtration would not permanently remove the cloud from either the high or low pH samples. At pH 5.0 none of these problems was encountered.

Attempts to use citric acid as a buffer were unsuccessful due to the preferential chelation of the aluminum ion by citrate. Therefore, if other products were to be analyzed by the aluminum complex method, sufficient aluminum must be added to satisfy the chelating potential of the entire system.

A. Height and Diameter Study

Results of this study for strain 873 are presented in the bar graphs, Figures 1 through 6. The numerical results for all strains are given in Tables 1 and 2.

The Folin-Denis reacting materials expressed as rutin equivalent per 100 g. of fresh asparagus increased with the height of the asparagus at harvest (Figure 1). They also increased with decreases in the spear diameter, indicating the Folin-Denis reactants are probably located in the surface area of the spears.

Ascorbic acid content is presented in Figure 2, both as ascorbic acid as determined directly and its computed rutin equivalent. The latter being obtained by multiplying the ascorbic acid content by a factor of 1.65 obtained by comparison of the slopes of the curves for the reaction of rutin and ascorbic acid with Folin-Denis. Ascorbic acid content per 100 g. of four-inch tip sections of asparagus increased with the maturity of the spear and with decreases in spear diameter. This is in agreement with Wolf's results on fresh asparagus showing ascorbic acid to be located in the cortex *(15)*.

The results of the rutin analysis (Figure 3) indicated a trend similar to that of ascorbic acid and Folin-Denis reacting substances. Stevenson *(12)* has also shown that the rutin content of asparagus increases with maturity. The increase in rutin per 100 g. of asparagus with decreases in spear diameter was confirmed in microscopic sectional studies. The epidermal layer of cross sections of asparagus stems is light in color and fluoresces blue under ultra violet illumination. When cross sections are soaked for a short period of time in dilute pH 5.0 buffered $AlCl_3$ solutions, the epidermis turns a brilliant yellow and fluoresces a yellow-orange under ultra violet illumination indicating the presence of rutin in the peripheral region of the asparagus spear.

TABLE 1

Folin-Denis ascorbic acid and rutin analyses for 5 strains of asparagus harvested at 3 heights above ground and segregated into 3 spear diameters (Results expressed on a fresh weight basis)

Sample		Total Folin-Denis reactants as rutin *(mg./100 g.)*					Ascorbic Acid *(mg./100 g.)*					Rutin from $AlCl_3$ method *(mg./100 g.)*				
Spear ht.	Spear dia.	Strain					Strain					Strain				
		873	309	500W	711	57	873	309	500W	711	57	873	309	500W	711	57
(inches)	*(inch)*															
<6	<3/8	197	200	396	235	311	37.8	37.2	49.7	47.6	48.7	24.5	23.2	45.7	33.4	51.1
	3/8–5/8	190	188	255	209	269	36.2	31.2	38.1	39.2	42.2	20.5	20.4	31.9	27.1	42.8
	>5/8	166	164	232	191	259	27.4	25.6	32.6	35.4	35.9	19.2	15.0	25.0	23.5	34.1
6–12	<3/8	293	313	403[1]	321	415	54.4	51.1	60.8	60.0	62.0	56.4	61.1	81.1[1]	63.8	88.7
	3/8–5/8	243	267	339	262	320	43.5	42.8	50.5	48.6	48.4	43.5	46.6	60.0	42.9	59.6
	>5/8	222	240	300	228	290	39.8	37.3	44.6	43.6	39.7	38.2	37.0	45.7	32.7	50.2
12–18	<3/8	392	395	477[1]	410	479[1]	72.0	59.4	72.8[1]	71.8	68.0[1]	84.8	87.3	99.8[1]	94.4	100.0[1]
	3/8–5/8	344	321	416[1]	353	385	63.8	52.0	62.4[1]	62.0	55.0	70.9	65.8	86.4[1]	78.2	80.3
	>5/8	287	286	361[1]	302	326	51.6	47.5	55.1[1]	54.9	47.2	54.3	52.3	65.5[1]	58.6	65.1

[1] Jars contained some yellow crystalline precipitate.

TABLE 2

Folin-Denis reactants other than rutin and ascorbic acid as rutin, titratable acidity and hydrogen-ion concentration of 5 strains of asparagus harvested at 3 heights above ground and segregated into 3 spear diameters
(Results expressed on a fresh weight basis)

Sample		Folin-Denis reactants as rutin other than rutin and ascorbic acid as rutin *(mg./100 g.)*					Titratable acidity *(m.e./100 g.)*					Hydrogen-ion concentration x 10^8				
Spear ht.	Spear dia.	Strain					Strain					Strain				
		873	309	500W	711	57	873	309	500W	711	57	873	309	500W	711	57
inches	*inch*															
<6	<3/8	110	115	268	123	180	1.49	1.78	2.24	2.00	2.12	293	300	398	337	272
	3/8–5/8	110	116	160	117	155	1.59	1.59	2.56	1.90	2.14	283	304	357	349	261
	>5/8	101	106	153	109	165	1.49	1.59	2.24	1.88	2.12	302	290	348	342	242
6–12	<3/8	146	167	221[1]	159	224	1.70	1.68	2.27	1.95	2.09	266	213	277	273	208
	3/8–5/8	128	150	196	139	180	1.56	1.63	2.24	1.93	1.97	245	196	259	272	200
	>5/8	118	142	180	123	175	1.56	1.59	2.24	1.88	2.22	269	188	272	283	200
12–18	<3/8	188	210	257[1]	198	267[1]	1.85	1.95	2.46	2.29	2.36	189	164	235	225	165
	3/8–5/8	168	170	227[1]	172	214	1.68	1.90	2.22	2.14	2.34	181	159	235	220	172
	>5/8	147	155	205[1]	152	183	1.70	1.70	2.24	1.97	2.29	199	200	236	231	172

[1] Jars contained some yellow crystalline precipitate.

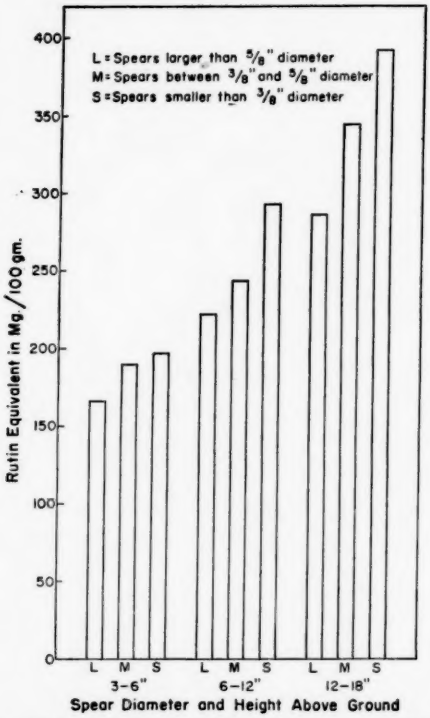

Figure 1. Rutin equivalent content of four-inch tips of asparague processed after harvest at different heights above ground—determined by Folin-Denis analysis.

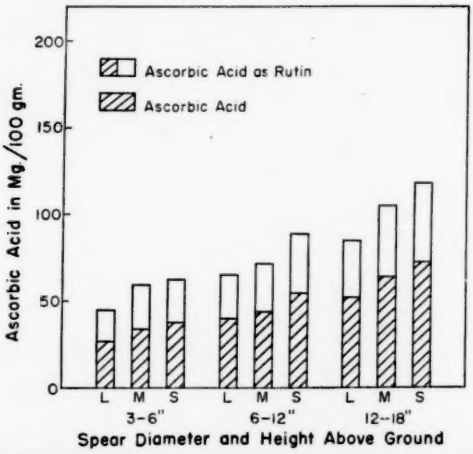

Figure 2. Ascorbic acid content of four-inch tips of asparagus processed after harvest at different heights above ground.

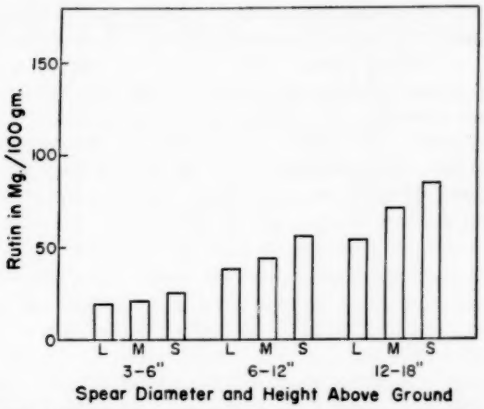

Figure 3. Rutin content of four-inch tips of asparagus processed after harvest at different heights above ground.

This method of staining also showed a very high concentration of rutin in the cladophylls.

The graph in Figure 4 represents the difference between the individual variables of the graph in Figure 1 and the sum of these variables (Figures 2 and 3). Thus, it indicates the changes in the Folin-Denis reacting materials (expressed as rutin) other than rutin and ascorbic acid as rutin. These materials showed the same general changes as the total Folin-Denis reactants, ascorbic acid and rutin. Numerous compounds may contribute to these

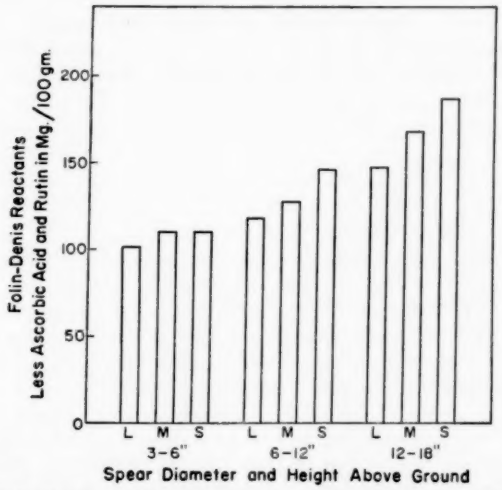

Figure 4. Folin-Denis reactants (expressed as rutin) other than rutin and ascorbic acid present in four-inch tips of asparagus processed after harvest at different heights above ground.

remaining Folin-Denis reactants. Therefore, the assumption implicit in this calculation is that their ratios are unaltered with changes in height or spear diameter.

Consistent changes in titratable acidity (Figure 5) with changes in spear diameter are not evident. The titratable acidity does show a tendency to increase with maturity. Culpepper and Moon *(2)* also reported an increase in titratable acidity with maturity in rapidly growing asparagus shoots. Consistent changes in hydrogen-ion concentration (Figure 6) with change in spear diameter likewise are not evident. However, the hydrogen-ion

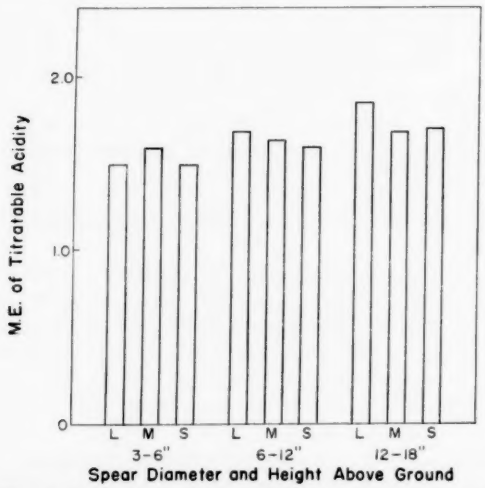

Figure 5. Milliequivalents of titratable acidity per 100 grams of four-inch tips of asparagus processed after harvest at different heights above ground.

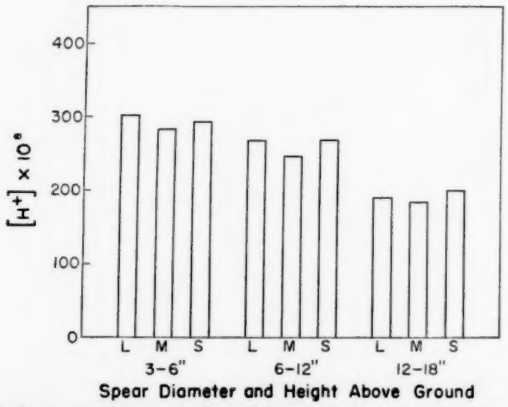

Figure 6. Hydrogen-ion concentration of four-inch tips of asparagus processed after harvest at different heights above ground.

concentration decreases as the spear matures. Thus an increase in titratable acidity is associated with a decrease in hydrogen-ion concentration. This may be due to a shift in the buffering system during growth or heating during retorting. The young meristematic region of the spear is high in proteins and amino acids. It is believed that these contribute greatly to the pH at which asparagus is buffered.

The generalizations noted are applicable to all 5 strains of asparagus studied (Tables 1 and 2). However, strains 500 W and 57 appear to be somewhat higher in rutin and Folin-Denis reacting materials than the other three. A review of weather conditions for the 4 or 5 days prior to harvest revealed that strains 500 W and 57 were harvested after periods of cool cloudy weather. The rutin content of a spear appears to be a function of the time that it is above ground prior to harvest. Thus, a spear grown during cool weather would have been above ground longer, have a slower rate of growth and have a higher rutin content than a spear of comparable height grown during warm, sunny weather. This factor may have contributed considerably to the difference between strains.

Tables 1 and 2 also show that 9 to 23% of the total Folin-Denis content was contributed by rutin and 23 to 33% by ascorbic acid, depending upon the height and diameter of the spear at harvest and the strain of asparagus. The Folin-Denis procedure is therefore inadequate as a quantitative measure of either rutin or ascorbic acid.

Tables 1 and 2 show that Folin-Denis reacting materials, ascorbic acid, rutin, and Folin-Denis reacting material other than rutin and ascorbic acid increase by a factor of 1.5 to 2 as the spear matures in height above ground from three to six inches to twelve to eighteen inches. The titratable acidity increases slightly and the hydrogen-ion concentration decreases by a factor of approximately 0.5.

B. Spear section study

Results of the analysis of 2-inch sections of medium diameter spears harvested at three heights above ground are presented on a fresh weight basis in Figures 7 to 12. These data are calculated on the same basis as the data for the height and diameter study.

The Folin-Denis reacting substances were highest in the tip sections and decreased rapidly in concentration in the base sections (Figure 7). The higher the spear above ground at harvest, the higher is the concentration of Folin-Denis reactants in comparable sections of the spear. This agrees with the results of the height and diameter studies. Figures 8 through 10 show that ascorbic acid, rutin and Folin-Denis reactants, other than ascorbic acid and rutin, change in a similar manner. Figure 11 shows that the titratable acidity per 100 g. of sample is highest in the tip sections and decreases in the base sections. Culpepper and Moon *(2)* found that titratable acidity was highest in the tip sections of asparagus spears.

The hydrogen-ion concentration is highest in the butt sections and decreases rapidly toward the tip of the spear and as the spear matures. The differences between the tip sections, however, are much less than those found for sections further down the spear. It is believed that the high protein and amino acid content of the tips contribute greatly to high pH values.

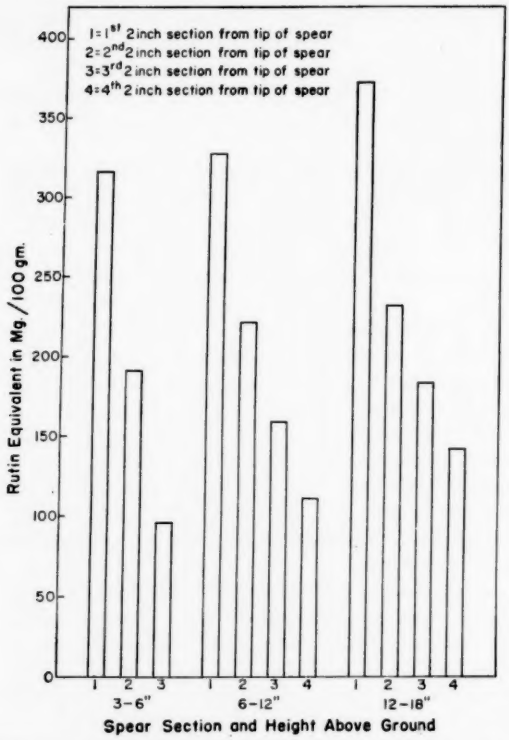

Figure 7. Rutin equivalent content of two-inch sections of asparagus spears processed after harvest at different heights above ground—determined by Folin-Denis analysis.

The average number of two-inch sections per 100 grams of asparagus spears harvested at different heights above ground is presented in Table 3. As the spear increased in height, the average number of sections decreased for the tip sections, remained almost constant for the second sections and increased for the third and fourth sections. These results indicate there is a tendency for the spears to become more uniform in diameter over their entire length as this dimension increases before harvest. Consideration of Table 3

TABLE 3

The average number of spear sections per 100 grams of ⅜–⅝ in. diameter spears of asparagus as influenced by the height of the spear at harvest

Two-inch spear section from tip downward	Height above ground		
	6 in.	6–12 in.	12–18 in.
1st.	25.3	23.5	21.5
2nd.	17.0	16.7	16.7
3rd.	11.9	12.9	13.3
4th.	.....	10.3	11.2

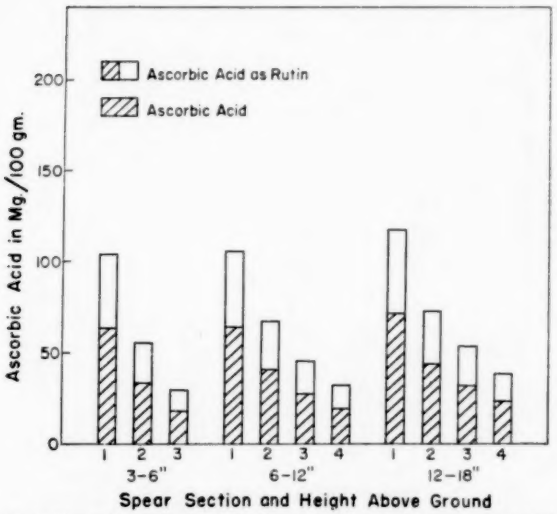

Figure 8. Ascorbic acid content of two-inch sections of asparagus spears processed after harvest at different heights above ground.

also shows that trends apparent on a weight basis do not necessarily exist on a per spear basis, since the surface area per unit weight is not constant.

The rate of growth of an asparagus spear increases as the spear increases in height above ground. Thus, any tip portion of a tall spear may be younger in time than a similar portion taken from a shorter spear. Yet results of the height and diameter study show that the tip portion of the taller spear has the highest concentration of the components measured. This may be due to differences in the length of growing region of the stalk. Culpepper and Moon *(3)* found that differences in the rate of elongation are caused principally by differences in the length of the growing region of the asparagus stalk and not by differences in the maximum rate of growth per unit length of growing zone. Furthermore, when the spear is increasing in height there is a formation

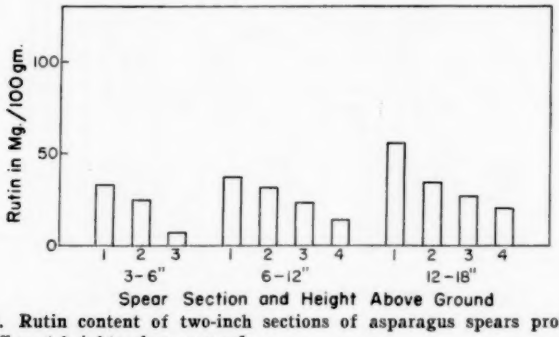

Figure 9. Rutin content of two-inch sections of asparagus spears processed after harvest at different heights above ground.

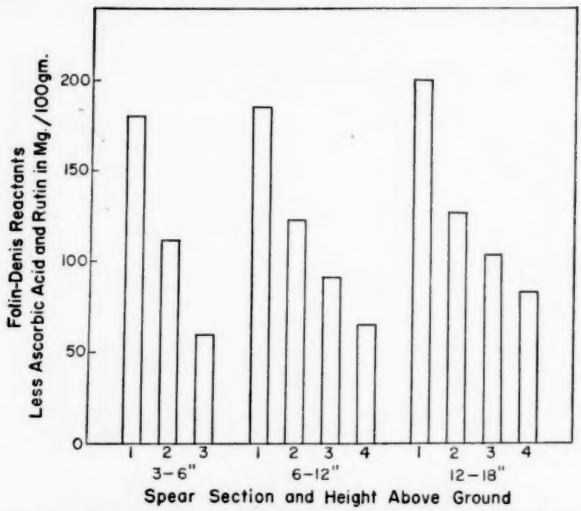

Figure 10. Folin-Denis reactants (expressed as rutin) other than rutin and ascorbic acid present in two-inch sections of asparagus spears processed after harvest at different heights above ground.

of many new meristematic regions prior to branching. The distribution of cladophylls should also be taken into consideration because of their high rutin content.

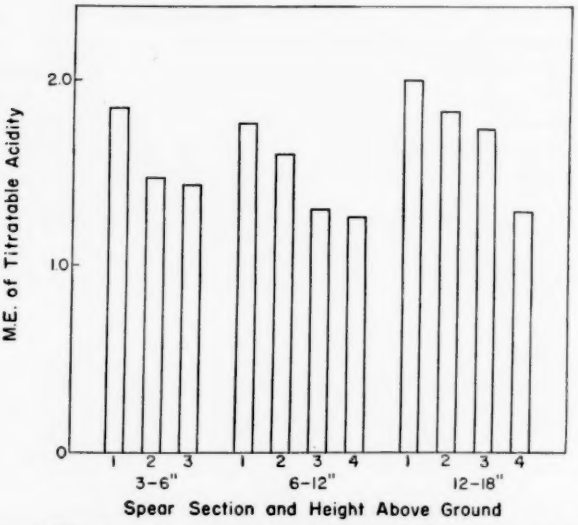

Figure 11. Milliequivalents of titratable acidity per 100 grams of two-inch sections of asparagus spears processed after harvest at different heights above ground.

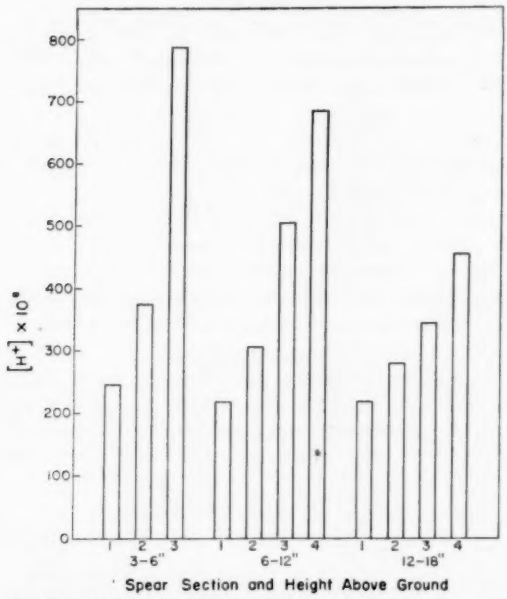

Figure 12. Hydrogen-ion concentration of two-inch sections of asparagus spears processed at different heights above ground.

C. Seasonal study

Results of the analysis of strains 500 and Mary Washington, harvested at weekly intervals, are presented in Table 4. For comparable height and diameter spears, these results agree closely with those obtained for the height and diameter study, except that the titratable acidity and hydrogen-ion concentrations are lower. No appreciable change in composition over the 3-week period is apparent, except that a slight increase in total Folin-Denis reactants, ascorbic acid and rutin is suggested. This may be due to the initiation of new meristems as asparagus tends to branch at lower heights as the season progresses.

TABLE 4

Composition of medium diameter 4-inch tip sections of asparagus, strains 500 and Mary Washington, harvested at weekly intervals for three weeks at 6 to 12 inches above ground during April 1956. Results expressed on a fresh weight basis.

Date harvested	Strain	Total Folin-Denis reactants as rutin (mg./100 g.)	Ascorbic acid (mg./100 g.)	Rutin by $AlCl_3$ method (mg./100 g.)	Titratable acidity (mg./100 g.)	Hydrogen-ion concentration x 10^8
14th	500	258	50.3	45.2	1.50	220
14th	M.W.	248	49.8	42.6	1.44	219
21st	500	284	54.0	44.5	1.70	200
21st	M.W.	263	50.7	41.7	1.50	200
28th	500	292	53.6	52.2	1.56	207
28th	M.W.	283	53.6	46.3	1.50	208

SUMMARY

Five strains of asparagus were harvested at three heights above ground, sorted into three diameters, processed in glass and analyzed for Folin-Denis reacting materials, ascorbic acid, rutin, titratable acid and pH.

Folin-Denis reactants, ascorbic acid, and rutin increased on a weight basis with height of spear above ground, and with decreases in spear diameter. pH, titratable acidity, increased with increase in height of spear above ground but did not change with spear diameter.

Analyses of spears harvested at 3 heights above ground and cut into 2-inch sections showed that on a fresh weight basis Folin-Denis reacting materials, rutin, ascorbic acid and titratable acidity were in highest concentration in the tip and decreased in concentration toward the base of the spear. The pH was also highest in the tip sections and decreased toward the base.

Acknowledgment

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STUDIES OF PROCESSED ALL-GREEN ASPARAGUS. II. THE EFFECT OF POST-HARVEST STORAGE AND BLANCHING VARIABLES UPON THE CHEMICAL COMPOSITION OF PROCESSED ASPARAGUS[a]

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Numerous studies on the influence of preparatory and processing variables on the loss of ascorbic acid in canned asparagus have been conducted *(3, 4, 5, 11)*. Wagner *et al. (11)* reported a loss of 6–11% in a water blanch at 150–190° F. Lamb *et al. (5)* reported excellent retention of vitamin C in canned asparagus which had been steam blanched. Other workers *(6, 9)* have studied the influence of storage temperature on the vitamin C concentration of fresh asparagus and related vegetable products.

Few reports have been published on the influence of postharvest storage and the preparatory operation used prior to canning on the flavonoid and organic acid content of asparagus. Schweigart and Kellner *(8)* reported that samples of asparagus stored at various temperatures showed an increase in acidity which was greater the higher the storage temperature. Stevenson *(10)* found a loss of rutin of 12.1, 21.9 and 31% in asparagus when the stalks were dried in an oven at 70, 100 and 110° C., respectively.

Asparagus harvested for canning purposes is subject to considerable variation in handling practices prior to and following its arrival at the cannery. It is presently hand cut and the spears are placed along the rows in small piles. A pick-up cart follows the cutters collecting these small piles for transportation to a cutting shed. The time between the cuttting and collecting operations varies, resulting in the asparagus being exposed to strong sunlight, high temperatures and/or hot dry winds for varying periods.

Upon arrival at the cutting shed, the spears are placed on conveying equipment which washes and cuts the spears to a uniform length (usually 7 inches). They are then placed tips up and tightly packed in 40-pound lug boxes which are stacked and generally hydrocooled by continual or intermittent spraying. At this point a time lapse often occurs before the boxes are loaded and hauled to the cannery. Upon arrival at the cannery the asparagus may be run through the plant immediately or stored temporarily. Whenever storage is necessary the boxes may be hydrocooled by continual or intermittent spraying or moistened and placed in cold storage at about (40° F.). The handling practices may also vary during the processing as the spears may be blanched with either water or steam.

Considerable change in concentration of certain constituents may occur between harvest and canning as a result of the variation in handling practices. The following study was carried out to determine their order of magnitude.

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EXPERIMENTAL

A. Sample preparation

Asparagus used in this study was obtained from the plots maintained on the University campus. It was harvested prior to 8:30 a.m. and brought without delay to the processing laboratory. The spears were immediately spray washed with water in the washing section of a Food Machinery and Chemical Corporation cup-down lye peeler. Following washing, the asparagus was handled according to the particular experimental design under study. These are described in detail in following sections.

Glass was used as the packaging container in these studies to avoid metal contaminaton of the product which would interfere with the rutin analyses. Stainless steel knives and trays and lacquered lids were also used. A weighed amount of asparagus and a measured amount of distilled water were used in all cases. This varied somewhat according to the size of jar used. All jars were steam jet closed. Then they were heat processed for 16 minutes at 248° F. with 10 p.s.i.g. over-riding air pressure following a 5-minute come-up period and pressure cooled to 150° F. F. with 25 p.s.i.g. over-riding air pressure. Upon removal from the retort, they were shaken to macerate the tissues and then allowed to cool to air temperature.

1. Storage study. Spears of asparagus strain 57 were harvested at heights of 8 to 12 inches above ground. After washing, the entire lot was size graded with respect to diameter of stalk at 4 inches from the tip. Those grading between $\frac{3}{8}$ to $\frac{5}{8}$ inch were selected and trimmed to a standard length of 8 inches measured from tip to butt.

Thirty-five lots of 150 g. each were weighed out and used as follows: 5 lots serving as controls were processed immediately in glass jars (303) with 300 ml. of distilled water; 10 lots were placed on a stainless steel screen which was placed on bare ground and exposed to the mid-day sun; 10 lots were placed on trays in an incubator room operating at 63° F. at a low relative humidity; and 10 lots, after binding each with string, were placed butt down in a lug box indoors and occasionally sprayed with water to simulate cannery storage conditions. At varying time intervals 5 lots from each treatment were withdrawn, reweighed, cut into one-inch segments, and processed in a manner identical to the controls.

2. Steam blanch study. Spears of asparagus strain 711 were harvested at heights of 6 to 12 inches above ground. After washing, the entire lot was trimmed to a standard length of 4 inches measured from tip to butt and then size graded. Spears measuring between $\frac{3}{8}$ to $\frac{5}{8}$ inch at the butt were used in this test.

Sixty-five lots of 75 g. each were weighed out and used for preparation of the following samples. Five lots were processed without steam blanching as controls. Remaining lots were exposed as a single layer to an atmosphere of moist steam at 150°, 175°, or 195° F. for 1 or 3 minutes and cooled in a water bath for 1 or 3 minutes in groups of 5 lots for each treatment. The cooling indicated above was accomplished by placing each of the 5 lots comprising a treatment in a separate container holding one liter of distilled water for the times indicated. Each lot was drained for 3 minutes on a stainless steel screen, cut into one-inch segments and processed in 8-ounce glass jars with 125 mls. of distilled water as described.

3. Water blanch study. Spears of asparagus strain 57 were harvested at heights of 6 to 10 inches above ground. Following the customary washing treatment they were trimmed to a standard length of 4 inches measuring from tip to butt and then size graded as above.

Five 100-g. lots were immediately processed as controls. Another five 100-g. lots were blanched separately in one gallon of boiling tap water for 3 minutes. Each lot was allowed to drain 3 minutes and then processed in glass jars (303) with 350 ml. of distilled water in the standard manner.

B. Analytical procedures

Forty-eight hours prior to analysis the glass jars were heated in an atmosphere of steam at 190° F. for 15 minutes, shaken while hot to further complete the maceration of tissues, and allowed to cool in air. It is assumed that a chemical equilibrium of the contents of the jar exists at this stage, and that one ml. of a filtrate prepared as described later equals one gram of total contents. Results are computed to an original fresh weight basis on this assumption. Values reported are the average of 5 replicate analyses.

1. The vacuum on each jar was taken with a Beidenberg F.I.R.A. gauge for canned products. The results are reported as true vacuum at room temperature, and were computed from the observed readings by the slide rule furnished with the instrument.

2. The pH value of an aliquot portion of the jar content was determined with a Beckman Model H2 pH meter.

The remainder of each jar was then filtered by suction to remove fibrous tissues using Hyflo Supercel in small amount as a filter-aid. The first 25–50 ml. portion through the filter was discarded in every case.

3. Aliquot portions of the clear filtrate were analyzed for titratable acidity, ascorbic acid, Folin-Denis reactants, and rutin.

The methods used for determining these constituents were identical to those described in a previous paper *(2)*.

RESULTS AND DISCUSSION

A. Storage study

The change of weight of the various samples during storage was recorded and is shown in Figure I, 1. These data were used to convert observed results to results expressed on a fresh weight basis before treatment. The difference in the initial chemical composition between the samples stored in the sun and the other two treatments resulted from starting this portion of the test at a later date.

The samples stored at room temperature and sprayed occasionally with water increased in weight while those stored under dry cool conditions and in the sun lost weight. Bisson *et al. (1)* and also Scott and Kramer *(9)* have reported an increase in weight and a decrease in per cent total solids of asparagus spears having a source of moisture during storage.

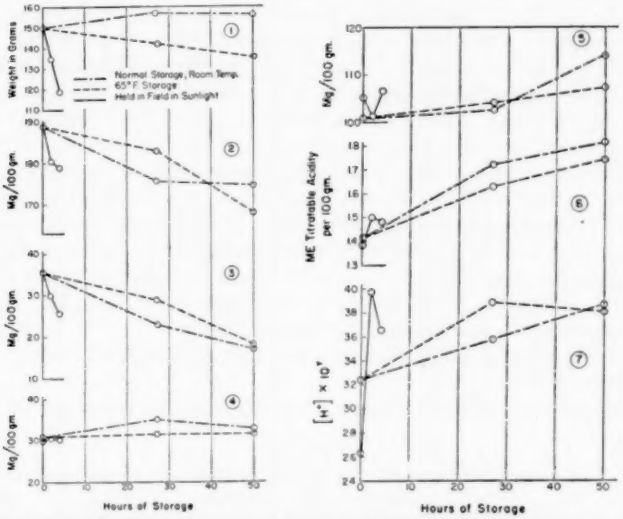

Figure I. The influence of various storage conditions on the weight or constituents present in all-green asparagus: 1. weight, 2. rutin equivalent as determined by Folin-Denis analysis, 3. ascorbic acid, 4. rutin, 5. Folin-Denis reactants other than ascorbic acid and rutin, 6. milliequivalents of titratable acidity, 7. hydrogen-ion concentration.

All analyses were conducted on samples processed in glass after the designated storage periods and results are presented on the original sample weight of 150 gms.

The concentration of both the Folin-Denis reactants and ascorbic acid decreased under all the conditions of storage tested. These results are shown in Figure I, 2, 3. The Folin-Denis data is expressed as rutin. Four hours of holding in the sun at mid-day resulted in approximately the same percentage decrease as storing the product for 20 hours at room temperature with occasional spraying or storing it for 32 hours at 65° F. and low relative humidity. The rate of change of these constituents for different storage conditions is quite variable, which is according to expectations as the respiratory activity undoubtedly is quite different under each storage condition tested.

The rutin concentration, Figure I, 4, remained practically unchanged in the samples stored under low humidity at 65° F. and in the sun, whereas a slight increase occurred in the asparagus stored under moist conditions at room temperature. Spears stored under the latter conditions increased in length; results indicate that this may be accompanied by a small increase in rutin.

Substances other than rutin and ascorbic acid reacting with Folin-Denis reagent appear to increase, Figure I, 5, in asparagus stored at low relative humidity at 65° F., and in that stored under moist conditions at room temperature. The trend is not quite so clear cut in asparagus stored in the sun where an initial decrease was followed by an increase of these substances. This is probably due to the difference in the rate of loss of ascorbic acid and production of other reacting compounds.

Titratable acidity increased with length of storage under all the conditions of the tests with the rate of increase being roughly proportional to temperature of storage. The hydrogen-ion concentration, on the other hand, does not show the same uniform relationship. Asparagus stored under low relative humidity at 65° F. and in the sunshine during mid-day first shows an increase in hydrogen-ion concentration which is then followed by a decrease, whereas the asparagus stored under moist conditions at room temperature shows a steady increase in hydrogen-ion concentration for the duration of the test. These differences may be due to the type of respiration occurring in turgid as compared to flaccid spears. It has been shown that the proteins and amino acids are catabolized during the respiration of asparagus, particularly under anaerobic conditions *(7)*. If these contribute materially to the buffer system eexisting in the spear, the buffering point would be lowered during storage as respiration continues. This would be evidenced by a lowering of the pH without equivalent increases in the titratable acidity.

The true vacuums in the glass jars of asparagus prepared from the storage samples are shown in Table 1.

A decrease occurred in the jars prepared from the asparagus held under

TABLE 1

Influence of storage treatment on true vacuum in glass jars of processed asparagus. Average of 5 replicates

	Storage treatment; hours at								
	65° F. at low R. H.			Room temp. with occasional spraying			Sunshine at mid-day		
	0	27	50	0	27	50	0	2	4
True vacuum	19.2	19.5	19.8	19.2	18.4	18.0	18.5	21.4	23.2

TABLE 2

Influence of blanch times and temperatures and length of water wash on certain chemical constituents of processed 4-inch tip section of asparagus strain 57, harvested April 26, 1956
(Based on fresh weight)

Temp.	Blanch time	Length of water wash	True vacuums	Total Folin-Denis as rutin	Ascorbic Acid	Rutin by $AlCl_3$ method	Folin-Denis reactants as rutin other than ascorbic acid and rutin	Titratable acidity	Hydrogen-ion conc. x 10^6
	(min.)	*(min.)*	*(inches)*	*(mg./100 g.)*	*(mg./100 g.)*	*(mg./100 g.)*	*(mg./100 g.)*	*(m.e./100 g.)*	
0	0	0	16.2	264	48.7	45.5	138	1.47	156
150	1	1	17.7	258	49.0	44.7	133	1.53	179
150	1	3	17.4	269	51.6	45.7	138	1.64	202
150	3	1	20.0	247	47.6	43.5	125	1.53	181
150	3	3	20.8	245	47.2	41.1	126	1.56	200
175	1	1	23.3	228	44.7	41.0	113	1.53	173
175	1	3	23.3	246	47.4	44.6	123	1.57	152
175	3	1	25.1	238	44.6	44.3	120	1.53	175
175	3	3	26.0	235	45.0	44.4	116	1.56	187
195	1	1	25.7	233	45.0	43.9	115	1.47	202
195	1	3	25.8	228	44.4	42.8	112	1.50	207
195	3	1	25.9	243	43.6	47.2	123	1.62	207
195	3	3	25.8	233	43.1	43.1	119	1.53	202

moist conditions at room temperature. As already noted these spears increased in both length and weight which apparently results in a greater amount of dissolved and occluded gases in the tissues at time of processing. The spears of asparagus stored in sunshine during the mid-day hours lost considerable weight and turgidity which would be accompanied by a decrease in occluded air. Thus, the true vacuums of the jars reflected the influence of test conditions on the amount of occluded and dissolved gases present in the spear at processing.

B. Steam blanch study

Results of the analyses obtained from the steam blanch study are presented in Table 2. Compensation for any change in weight occurring during blanching, washing and draining was neglected during the preparation of the samples and in the computations used to derive the data. These losses were considered negligible as a study of 10–100 g. samples steam blanched at 195° F. for 3 minutes and water washed for 3 minutes showed a 2% loss in weight during blanching which was completely recovered during the washing.

In all cases, 75 g. of fresh material was blanched, washed and drained and then processed in 8-ounce baby food jars with 125 ml. of water. Although the results are not entirely consistent, the total Folin-Denis reactants, ascorbic acid, rutin and Folin-Denis reactants other than rutin and ascorbic acids showed a tendency to decrease as the temperature and time of blanch and the length of water wash were increased. The rutin concentration was least affected while the materials reacting with the Folin-Denis reagent other than rutin and ascorbic acid were affected to the greatest extent.

The titratable acidity increased slightly as did the hydrogen-ion concentration as the asparagus was subjected to a heat treatment. The degree of heating does not appear to cause any large changes in the observed results over the range of temperatures studied.

Visual observations of the wash water during the preparation of the samples showed an increase in the color of the wash water with an increase in blanch temperature and length of water wash. Blanching at 150° F. did not appear to impart any color to the wash water.

The most obvious effect of increased blanch time and temperature is reflected in the increase in true vacuum. A 3-minute blanch at the lower temperatures is more effective in removing dissolved and occluded air from the tissues than a 1-minute blanch. At the higher temperatures the effectiveness of length of blanch is less pronounced. The differences in vacuums of the processed product obtained by the various blanching treatments would be much greater for commercially processed products as the ratio of product to brine would be much larger.

C. Water blanch study

Results obtained for the water blanch study are presented on the original fresh weight basis in Table 3. As in the steam blanch study the change in weight during blanching was neglected in the calculation of the data and it was assumed that 150 g. of asparagus was added to each jar under study. A study of 10–100 g. lots of asparagus blanched in tap water for 3 minutes at 212° F. and drained for 3 minutes showed an average increase in weight of

TABLE 3

Influence of a water blanch at 212° F. for 3 minutes on the chemical composition of processed 4-inch tip sections of asparagus strain 57 (Based on fresh weight)

Treatment	True vacuums	Total Folin-Denis as rutin	Ascorbic acid	Rutin by $AlCl_3$ method	Folin-Denis reactants as rutin other than rutin and ascorbic acid	Titratable acidity	Hydrogen ion conc. x 10^8
	(inches)	*(mg./100 gm.)*	*(mg./100 gm.)*	*(mg./100 gm.)*	*(mg./100 gm.)*	*(meq./100 gm.)*	
Control	20.5	295	54.0	48.2	158	2.04	200
Water blanch	25.4	224	41.0	37.4	119	1.44	132
Per cent loss	.....	24.1	24.1	22.4	24.1	29.4	34.0

2.3 g. Thus the increase in moisture content more than compensated for the loss of soluble solids. Under the conditions of this study such a change in weight would have resulted in less than a 1% change in the numerical values of Table 3.

As might be expected, a very considerable loss was found in the constituents under study because of their water soluble characteristics. For example the concentration of rutin and ascorbic acid was reduced 22.4 and 24.1%, respectively. This treatment approximated the time-temperature relations formerly used in blanching asparagus for canning.

A differential rate of leaching of the various water soluble constituents is indicated. The higher percentage loss of hydrogen-ion concentration over titratable acidity may be partly due to the differential leaching of the components of the buffering system.

SUMMARY

Cut asparagus was steam blanched for various lengths of time, water washed, processed in glass and subsequently analyzed for Folin-Denis reactants, ascorbic acid, rutin, titratable acidity, and pH. Asparagus was also subjected to a water blanch at 212° F. for 3 minutes processed and analyzed as before. The steam blanch treatments resulted in only small changes in the concentration of the constituents determined. The water blanch treatment, however, reduced the Folin-Denis, ascorbic acid and rutin concentrations 24%, 24% and 22%, respectively; the titratable acidity and hydrogen-ion concentrations also decreased.

The influence of postharvest storage upon the composition of asparagus processed in glass was determined by storage under various conditions. Generally, storage was reflected by a decrease in Folin-Denis reactants and ascorbic acid and an increase in titratable acidity and hydrogen-ion concentration in the processed product.

Acknowledgment

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THE MICROBIOLOGY OF FRESH AND IRRADIATED BEEF[a]

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When fresh beef is allowed to remain at 0–2° C. in a moist environment it will spoil within a few days as a result of bacterial growth. The onset of spoilage is detected by the appearance of surface slime, discoloration of the meat pigment, and the production of a musty odor. Many investigators have studied the flora responsible for this spoilage *(4)*, and the predominant organisms had been generally classified as *Achromobacter* species. However, as pointed out by Kirsch *et al. (5)*, current methods of classification would identify many of these as *Pseudomonas* species. These latter authors studied the flora of ground beef and found members of the genus *Pseudomonas* to be the most common organisms, with a substantial number of lactobacilli and some micrococci also occurring in significant numbers. *Pseudomonas geniculata* and a group that differed from *P. geniculata* only by their failure to liquefy gelatin were the most frequently encountered species, with lesser numbers of *Pseudomonas fragi* and *Pseudomonas rugosa.*

Because of the rapid spoilage of fresh beef under normal conditions, satisfactory methods of preservation are sorely needed. One of the most promising new methods appears to be the use of high energy ionizing radiations. However, if fresh beef is irradiated at dosages that are required for complete sterilization, undesirable odors and flavors are produced. Thus, the more promising application of irradiation to beef preservation would appear to be through the use of lower levels of irradiation to extend the refrigerated shelf-life of prepackaged cuts *(7)*. The amount of irradiation that would be required to accomplish this objective must be adequate to destroy most of the microbial flora that can multiply at the usual refrigeration temperature, and this level must be below the amount that induces significant changes in odor and flavor.

EXPERIMENTAL METHODS

The initial phase of this investigation consisted of a survey of the bacteria responsible for the spoilage of fresh beef held at low temperatures. Samples of sliced beef round were placed in sterile petri dishes containing 3–5 ml. of sterile distilled water and incubated at 2° C. in a slanting position. After spoilage became evident, the samples were quantitatively plated in TY agar (1.0% tryptone, 0.5% yeast extract, and 1.5% agar) and in APT agar *(3)*. The plates were incubated at 25° C. for 5 days.

Representative colonies were isolated and identified according to methods listed in the *Manual of Methods for Pure Culture Study of Bacteria (2)*. The basal medium for most of the physiological tests on pseudomonads was 0.1% tryptone and 0.1% yeast extract. Georgia and Poe's asparagine agar was used to detect fluorescein production, and Gessard's agar for determining pyocyanin production *(8)*. The basal medium for the study of other microorganisms was 1.0% tryptone, 0.5% yeast extract, 0.5% K_2HPO_4, 0.5% NaCl, and 0.05% glucose.

Radiation resistance studies were conducted on suspensions of pure bacterial cultures and on inoculated packaged meat samples. The samples were irradiated with gamma rays

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from a 561-curie cobalt-60 furnace that initially emitted approximately 1600 rads per minute, with a decay rate of approximately 1 per cent per month. The radiation chamber was refrigerated at approximately 5° C., and all samples were held in crushed ice before and after irradiation. Irradiated samples were usually plated within 30 minutes after removal from the cobalt furnace.

All radiation dosages are reported in rads, as defined in the National Bureau of Standards *Handbook 62.* The rad has been officially adopted by the Quartermaster Corps. One rad is equivalent to 100 ergs of energy absorption per gram in material of unit density; thus, one rad is equivalent to 100/93 rep.

RESULTS

Bacterial flora of refrigerated fresh beef. Before incubation most of the fresh beef samples had moderate numbers of bacteria, the majority of which were gram-positive rods. However, after incubation in moist chambers at 2° C. until spoilage became evident, the predominant flora consisted of gram-negative rods. A physiological study was made of 39 representative cultures isolated from spoiled fresh beef. They were all non-pigmented, gram-negative, highly aerobic rods having polar flagella. Thus, they all belonged to the genus *Pseudomonas* as presently defined *(1).*

All strains produced acid but no gas from glucose, and none produced acid from fructose, sucrose, or glycerol. Only one strain reduced nitrate to nitrite, and all but one strain grew at 0° C. About half of the strains liquefied gelatin. However, when these strains were retested a year later some of them had lost the ability to liquefy gelatin. The gelatin-liquefying strains could best be classified as *Pseudomonas geniculata.* The other strains might be classified as *Pseudomonas rugosa,* but are indistinguishable from *P. geniculata* except for the gelatin test. One culture was identified as *Pseudomonas fragi.* Two strains produced fluorescein and grew at 37° C. One of these liquefied gelatin and the other did not, thus being similar to *Pseudomonas jaegari* and *Pseudomonas convexa,* respectively. Thus, about 90% of the isolates were either *P. geniculata* or differed from it primarily by an inability to liquefy gelatin.

Radiation resistance of pseudomonads. Tests on suspensions of pure cultures demonstrated that the pseudomonads are among the most radiation sensitive of all bacteria. Two factors were found to affect significantly the radiation resistance of these cells: the population density and the suspending medium used during irradiation. As shown in Table 1, regardless of the suspending medium, dense cell suspensions (having more than 3×10^7 cells per ml.) were more resistant to irradiation. Their resistance was fairly constant at population levels below 3×10^6 cells per ml.

In order to explore further the effect of initial population density, small numbers of viable cells were irradiated in the presence of large numbers of either heat-killed or radiation-killed cells from the same culture. In neither instance were the viable cells protected. Also, high populations of viable cells of other bacterial species (a *Pediococcus* and a *Staphylococcus*) failed to protect small numbers of pseudomonads. Conversely, high viable populations of the *Pseudomonas* failed to increase the resistance of a strain of *Streptococcus faecalis* to gamma radiation.

Many factors that have been reported to influence the radioresistance of other microorganisms were found to have little or no effect on the *Pseudomonas.* These include—reduced oxygen tension during radiation, pH

TABLE 1

Resistance of Pseudomonas geniculata to gamma radiation in various suspending media

Initial population *(log)*	Dosage (rads) for 99% destruction			
	TY broth	Meat juices	Phosphate buffer	Culture filtrate
8.5	23,000	34,000	17,500	30,000
7.5	20,500	22,000	9,500	11,000
6.5	6,500	16,000	6,500	6,500
4.5	6,500	15,000	6,500	6,500

values of the suspending medium between 5 and 8, the age of the culture, and irradiation in the presence of free radical acceptors (ascorbic acid, nitrite, glutathione, cysteine, thioglycollate, and thiourea). However, as shown in Table 1, meat juices afforded some protection, particularly to smaller concentrations of the cells. The protective factor was heat stable and water soluble, and thus could be found in the juices from cooked meat. The washed particles of cooked meat had no activity.

It has also been found that ferrous salt autoclaved in TY broth or in the presence of a reducing sugar such as glucose form a protective factor. This is illustrated in Table 2. If the glucose and Fe^{++} were sterilized separately, either by autoclaving or by filtration, there was no protective effect.

Irradiation of inoculated meat samples. Samples were taken from the centers of carefully trimmed fresh beef sirloin, inoculated with 10^3–10^4 pseudomonads per gram, sealed in cellophane pouches, and irradiated. After irradiation at different dosages, one set of samples was immediately quantitatively plated and the other samples were stored at 2° C. in a humid atmosphere. A dosage of 44–66,000 rads was found to be sufficient to kill virtually all of the pseudomonads. As measured by bacterial population figures (see Table 3), these irradiated meat samples had a shelf-life at 2° C. that was 4-5-fold that of the unirradiated controls. As would be expected, the irradiated samples eventually spoiled due to bacterial action. In contrast to unirradiated beef, the spoilage was characterized by a sour odor and flavor. The bacteria causing this spoilage were non-motile, non-sporeforming, gram-positive rods that were catalase-positive, facultative and psychrophilic.

TABLE 2

Effect of ferrous salts on the radiation resistance of Pseudomonas geniculata

Suspending medium[1]	Dosage (rads) for 99% destruction
TY broth	5,500
TY broth + 10^{-4} M Fe^{++}	5,000
TY broth + 10^{-3} M Fe^{++}	17,500
TY broth + 10^{-2} M Fe^{++}	24,000
1% glucose	6,500
10^{-3} M Fe^{++}	6,500
1% glucose + 10^{-3} M Fe^{++}	20,500

[1] 0.5% sodium citrate was included in all of these suspension media and all were sterilized by autoclaving.

TABLE 3

Effect of irradiation on the bacterial count of fresh beef inoculated with moderate numbers of pseudomonads [1]

Days of storage at 2° C.	Bacteria per gram of meat			
	No irradiation	22,000 rads	44,000 rads	66,000 rads
0	$<10^4$	$<10^3$	<10	<10
5	2×10^6	4×10^4	50	160
7	4×10^6	1×10^8	2×10^3	130
14	8×10^9	2×10^9	2×10^7	1×10^4
23	4×10^{10}	1×10^{10}	2×10^9	8×10^6

[1] The inoculum consisted of approximately 10^3 cells of *Pseudomonas geniculata* per gram of meat. Total counts were determined in TY agar and APT agar. The counts were not significantly different in these media with any sample.

Because of the repeated predominance of the gram-positive organisms after incubation of irradiated meat, five samples of fresh unirradiated beef from five different retail stores were examined. In three of them the gram-positive rods were predominant, and in the other two samples the flora were about evenly divided between these organisms and pseudomonads.

A detailed taxonomic study of these gram-positive rods indicated that they were a homogeneous species, very similar to *Microbacterium thermosphactum* described by McLean and Sulzbacher *(6)*. In addition to the characteristics listed above, they fermented glucose, mannose, fructose, maltose, sucrose, trehalose, and mannitol. They did not ferment xylose, arabinose, galactose, lactose, raffinose, inulin, glycerol, or sorbitol. They all grew at 2° C. and 30° C. but not at 37° C. They grew in the presence of 6.5% salt, but not in the presence of 10.0% salt. They hydrolyzed esculin but did not hydrolyze sodium hippurate, gelatin, starch, or arginine. They did not reduce nitrate nor produce CO_2 from glucose. They produced acetoin from glucose, gave a final pH of 4.5–4.9, were homofermentative, and produced L (+) lactic acid from glucose fermentation.

These organisms differed from the published description of *M. thermosphactum* primarily by their failure to ferment xylose, arabinose, and lactose, and their failure to produce CO_2 from glucose. However, two strains of *M. thermosphactum* were obtained from Dr. Sulzbacher, and these two strains also failed to ferment xylose, arabinose, or lactose, and did not produce significant amounts of CO_2 from glucose.

One other unique group of microorganisms were occasionally noted on irradiated beef, but they are of doubtful significance. These were gram-negative cocci, in pairs, that did not ferment any sugars or polyalcohols, nor did they hydrolyze gelatin, starch, or esculin. They were catalase-positive and grew at 2° C. and 30° C. but not at 37° C. No conclusions have been reached concerning their taxonomic identification.

DISCUSSION

The present work illustrates the influence of environmental conditions on the type of microorganisms that ultimately spoil a product. Fresh beef has an initial flora that is predominantly made up of gram-positive, catalase-positive,

non-sporeforming rods. After storage at 2° C., pseudomonads become predominant and cause spoilage. Irradiation of the beef with a moderate dosage of gamma rays readily destroys the pseudomonads but some of the gram-positive flora survive and multiply slowly at 2° C. It is quite probable that at higher storage temperatures a much wider variety of spoilage organisms would become evident.

The fact that the pseudomonads that grow with relative rapidity at low temperatures are destroyed by low doses of radiation and the surviving organisms grow much slower at these temperatures accounts for the fact that low levels of irradiation can markedly extend the shelf-life of fresh beef.

SUMMARY

Fresh beef stored at 2° C. in a humid atmosphere spoils rapidly due to the growth of *Pseudomonas geniculata* and related pseudomonads. However, these microorganisms are among the most sensitive to gamma radiation of all bacteria. In culture media, 6500 rads are adequate to kill 99% of moderate population levels. In meat juices, approximately 15,000 rads are required to kill 99% of similar cell concentrations. At high population densities, the dosage necessary to kill 99% may be increased to 23,000 rads in culture media and 34,000 rads in meat juices.

When fresh meat is inoculated with moderate numbers of pseudomonads, an irradiation dosage of 44–66,000 rads is sufficient to kill virtually all of the pseudomonads. The shelf-life of such beef at 2° C. may be extended as much as 4-5-fold. The irradiated beef eventually spoils bacteriologically due to the slow growth of a radiation-resistant group of organisms, apparently identical to *Microbacterium thermosphactum*. This organism was found to constitute a significant proportion of the flora of fresh beef before being overgrown by the pseudomonads during storage at 2° C.

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NOTES AND LETTERS

EFFECT OF CARBONATED BEVERAGES ON BACTERIA

The presence and survival of bacteria in those carbonated beverages referred to as "soft drinks" present a question of public health significance as well as one of industrial importance. Not a great deal of work has appeared in the literature in recent years on this problem. Probably the most thorough piece of work of this nature was carried out by Shillinglaw and Levine *(2)*. These authors demonstrated that edible acids—such as phosphoric, citric, acetic, lactic, glycolic and tartaric in a concentration of 0.02 N, which is approximately that employed in many beverages—were effective germicides against *Escherichia coli* and *Salmonella typhosa*. Addition of carbon dioxide and sucrose increased the germicidal efficiency of these acids to a small degree. An excellent review of the literature pertinent to problems of this nature up to that date (1943) was presented by these workers.

More recently, Insalata *(1)* reported that carbonation at high pressures was effective in inactivating bacteria, yeasts and molds in bottled beverages while more time was required to inactivate at lower pressures. This worker, however, employed rather small numbers of microorganisms in his studies.

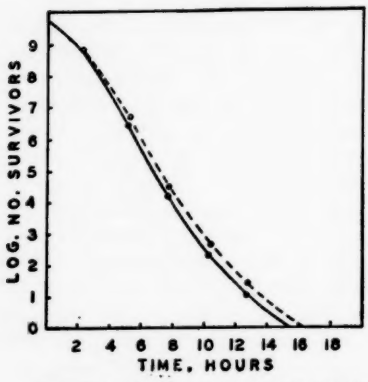
In both of the published works cited, artificially concocted systems were employed rather than actual commercial beverages. The purpose of the experiments reported in the present paper was to determine the effect of carbonated beverages *per se* on bacteria and to determine the influence of carbonation.

Four different brands of popular carbonated beverages were used and the results obtained from each experiment were nearly identical. *Escherichia coli, Micrococcus pyogenes* var. *aureus,* and *Salmonella enteritidis* were the test organisms. Numbers of bacteria were employed varying from several hundred to 6.5 billion per bottle of beverage. The bottled beverages were resealed after inoculation with their own caps by means of a Trinco Recap,[a] an instrument adapted for this purpose. Very little loss of carbonation resulted. In every case, after less than 24 hours of incubation at either 4° C., 25° C. or 37° C., all of the bacteria inoculated into each bottle appeared to have been killed when samples were plated onto suitable media.

Beverages employed had been carbonated with 3.5 volumes of CO_2 and demonstrated reactions of pH 2.5 to pH 3.0, with pH 2.5–2.8 being average. The pH remained unchanged even when the CO_2 was removed by boiling the beverages. With this knowledge, an experiment was then designed to determine the roles played by the CO_2 and the acidity in the killing of bacteria. Large numbers of *E. coli* and *M. pyogenes* variety *aureus* were inoculated into fully carbonated beverages and into the same beverages whose carbonation had been removed by boiling. Two different beverages were employed, both containing 3.5 volumes of CO_2 and having a pH of 2.5–2.8. However, one beverage was acidulated with phosphoric acid while the other contained citric acid. In every case, sterility had resulted at the end of 16 hours and the

[a] Trinco Industries, Inc., Chicago.

results obtained from both beverages and both organisms were nearly identical. Results obtained from *E. coli* are expressed in Figures 1 and 2 for beverages acidulated with phosphoric acid and citric acid, respectively.



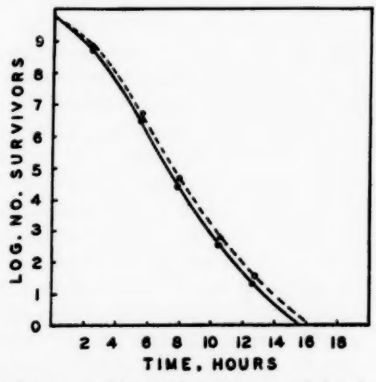


Figure 1. Killing curve for E. coli inoculated into a carbonated beverage acidulated with phosphoric acid. Solid line—fully carbonated; broken line—uncarbonated.

Figure 2. Killing curve for E. coli inoculated into a carbonated beverage acidulated with citric acid. Solid line—fully carbonated; broken line—uncarbonated.

In summary, it can be stated that carbonated beverages present a highly unfavorable environment for bacteria. Experimental data here presented indicate that the killing effect is derived mainly from the low pH involved and that the carbonation increased the efficiency of killing only slightly.

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OBSERVATIONS ON THE REGREENING OF LATE VARIETIES OF ORANGES

Toward the end of the period for commercial harvesting of late varieties of oranges (Valencia, Lue Gim Gong, and others) many of the fruits are characterized by a tinge of green color in the rinds *(1, 2, 3, 4)*. Most pronounced at the stem end, it decreases in quantity from there to the "equator". Green color is rarely found in the lower hemisphere of these fruits.

Oranges of this kind are said to have "regreened." The peculiar pattern of regreened oranges distinguishes them from fruits which are green because they are immature or because they have been completely shaded. Immature and shaded fruits are uniformly green in color, whereas the green pigment in regreened specimens is confined to the upper hemisphere. These regreened oranges are fruits which have first lost their green pigmentation during the colder months of the winter and then have become green again to a certain extent during the warm weather of late spring and early summer.

During late spring and early summer regreened oranges from Florida become conspicuous on both wholesale and retail markets. Casual inspection of orange crates and fruit bins indicates that not all of the fruits show the characteristic regreening. A certain percentage are characterized by a good orange color over the whole surface. This has been observed in the grove also. What has interested the senior author for a number of years is that regreened oranges seem to lack some of the internal quality possessed by full-colored oranges in the same lots, whether taken from market fruit bins or from the tree. The object of these investigations was to make a detailed study of the physiological characteristics of regreened and full-colored oranges.

MATERIALS AND METHODS

Six lots of Valencia oranges were purchased on the local retail market over a period of 3 years. Three lots were from California, the others from Florida. Florida oranges were purchased on June 25, July 22, and May 22 the following year. California oranges were purchased on August 29, June 25 and July 17, each on successive years. Advantage was taken of self-service facilities to select a dozen regreened and a dozen full-colored fruits at each purchase. In the laboratory 10 oranges of each type were paired so that they were comparable in size and shape, differing only in the manner in which their rinds were pigmented. Juice of each orange was analyzed separately for total soluble solids and total and ascorbic acids. Additional lots of fruits were shipped directly from Florida to Pittsburgh by Railway Express. These oranges had received no packing-house treatment. Three of the lots consisted of Valencia oranges from near Orlando; the other was Lue Gim Gong from 2 trees in Winter Garden. These lots, sorted upon arrival in the laboratory, were paired in the manner described. Since the oranges had not been graded and sized in the packing house, fruits were not as uniform in size as those graded and offered for sale on retail markets. Nevertheless, it was possible to pair a number of each type and to analyze relatively large samples.

Total soluble solids were determined by means of a specific gravity hydrometer. For total acid determinations an aliquot of the juice was titrated with 0.15 normal NaOH and the results were expressed as grams acid as citric. Ascorbic acid content was obtained by combining equal quantities of juice and 8% acetic acid and titrating with sodium 2,6-dichlorobenzenoneindophenol.

RESULTS

Table 1 presents results of the analyses of the Valencia oranges collected on the retail markets. It will be noted that regreened oranges tend, generally, to be lower in

both soluble solids and in ascorbic acid than fruits which have lost all green color. No consistent differences in total acids are apparent. Considering average values, full-colored Florida oranges had 31% more soluble solids and 29% more ascorbic acid than "regreened" fruits. Full-colored California oranges averaged 21.8% higher in soluble solids and 20.3% higher in ascorbic acid than the "regreened". Table 2 gives the results

TABLE 1

Total soluble solids and total and ascorbic acids in the juice of "regreened" and full-colored Valencia oranges collected on the market

State and date	Total soluble solids (%)		Total acid *(gm. per 100 ml.)*		Ascorbic acid *(mg. per 100 ml.)*	
	Regreened	Full-colored	Regreened	Full-colored	Regreened	Full-colored
Florida:						
6/25/52	9.3	13.4	0.61	0.61	21.5	26.2
7/22/52	13.5*	15.1*	0.64	0.61	24.6*	38.4*
5/22/53	8.9*	12.9*	0.79	0.86	34.3	37.7
Average	10.5	13.8	0.74	0.69	26.8	34.7
California:						
8/29/51	12.3	12.9	1.10	0.95	49.4	49.4
6/25/52	11.7	13.2	1.40	1.31	51.8	61.2
7/17/53	10.3*	13.0*	1.10	1.10	52.2	66.0
Average	11.4	13.0	1.20	1.12	51.1	58.8
Grand average	11.0	13.4	0.96	0.90	38.9	46.8

* Statistically significant difference.

on Lue Gim Gong oranges from Florida. Because of the relatively large number of fruits available for this test they were divided into 6 lots, each containing 5 regreened and 5 full-colored oranges. Data for average weight and volume of juice are presented to show how closely the pairs in each sample were matched. These results are similar to those in Table 1 in that again the regreened oranges in general contained lower soluble solids and smaller quantities of ascorbic acid than the full-colored fruits. When results are averaged, full-colored oranges contained 18.3% more soluble solids and 22% more ascorbic acid than the regreened fruits.

Results obtained from the Florida Valencia oranges collected from the groves in 1954, 1955 and 1956 are presented in Table 3. Each of the lots contained 10 to 12 regreened and 10 to 12 full-colored oranges. These results are similar to those previously presented in that, though there is some variation between samples of each lot, when all are averaged full-colored fruits show higher soluble solids and higher ascorbic acid content than regreened fruits.

DISCUSSION

Data presented here suggest that internal quality of regreened oranges may not be as high as that of fully-colored oranges harvested at the same time. It tends to corroborate the senior author's impression of this difference in quality as evidenced by taste tests. It is true that differences between

TABLE 2

Weight, juice volume, total soluble solids, total and ascorbic acids in the juice of "regreened" and full-colored Lue Gim Gong oranges, all from the same two trees (Picked May 26)

Sample	Weight (gm.)		Juice volume (ml.)		Total soluble solids (%)		Total Acid (gm. per 100 ml.)		Ascorbic Acid (mg. per 100 ml.)	
	Regreened	Full-colored	Regreened	Full-colored	Regreened	Full-colored	Regreened	Full-colored	Regreened	Full-colored
A	202	204	83	82	11.4	13.8	0.67	0.71	30.2	38.2
B	180	182	73	76	12.6	13.8	0.72	0.73	41.1	40.0
C	188	170	75	68	12.6	15.0	0.71	0.84	40.0	41.8
D	148	148	57	56	11.4	15.0	0.72	0.87	32.3	47.0
E	202	196	83	81	11.4	13.8	0.79	0.77	26.4	40.9
F	200	190	79	82	12.6	13.8	0.80	0.71	31.8	38.2
Average	186	198	75.3	74.4	12.0*	14.2*	0.73	0.76	33.6*	41.0*

* Statistically significant difference.

TABLE 3

Total soluble solids, total and ascorbic acids in the juice of Florida Valencia oranges collected in the grove

Date	Total soluble solids (%)		Total acid (*gm. per 100 ml.*)		Ascorbic acid (*mg. per 100 ml.*)	
	Regreened	Full-colored	Regreened	Full-colored	Regreened	Full-colored
5/4/1954	10.8	11.1	0.57	0.57	29.6*	34.8*
6/8/1955	13.3	14.2	0.70	0.73	36.3	37.3
5/29/1956	13.6*	14.5*	0.56	0.46	42.9	46.8
Average	12.9	13.2	0.61	0.55	36.2	40.6

* Statistically significant difference.

regreened and full-colored fruits were not always statistically significant. Significant differences were observed between soluble solids more often than between ascorbic acid content. Lack of statistical significance could be attributable to variability between samples. This was especially true in the fruits collected from the markets. The two different types of oranges appearing in the fruit bin of a retail store may not necessarily have been produced in the same grove. The Lue Gim Gong oranges, on the other hand, showed significant differences in both soluble solids and ascorbic acid. Harvesting was carried out by commercial pickers so that samples were randomized. Valencia oranges collected in the groves over a period of 3 years showed individual variability somewhat like those collected on the market. Statistical significance was shown in differences in soluble solids in only one year's collections. The same was true of ascorbic acid. The Lue Gim Gong oranges and one sample of Valencia oranges from the market were the only ones to show significant differences in both soluble solids and ascorbic acid.

Caprio *(3)* studied the problem of regreening in California oranges and reported that certain environmental factors are conducive to regreening of late oranges. This investigator observed that good color developed in oranges during years in which the average blooming date is April 5; regreening developed in years in which the average date of bloom was April 23. In other words, a definite relationship between late blooming and subsequent regreening is indicated. In the results reported in this paper lower quality in the regreened fruits could be attributed to the fact that these particular fruits resulted from a late bloom. If collections are made late in the commercial season for Valencia oranges, then the full-colored fruits will possibly have had sufficient time to accumulate higher soluble solids and to acquire a very sweet taste. Whatever the case, whether regreened oranges represent fruits which have regreened following a late bloom or whether they are late-bloom fruits which have not lost all of their green color, these poorly colored fruits are generally not as high in quality as fully colored fruits. It is possible, therefore, that lower quality in the regreened oranges which were purchased on the market may be attributable to a later bloom.

SUMMARY

Regreened and full-colored Valencia oranges from Florida and California were collected on the market and analyzed for total soluble solids and total and ascorbic acids. Although samples showed considerable variability, there was a tendency for the juice of full-colored fruits to be higher in soluble solids and in ascorbic acid than that of the regreened fruits.

Similar results were obtained with Valencia and Lue Gim Gong oranges shipped to Pittsburgh from Florida.

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Acknowledgments

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Food Research, **22** (2) 117 (1957) For the article entitled "Quantitative Aspects of Strawberry Pigment Degradation" the following corrections have been submitted:

Page 117, line 5 from end; also, page 118, line 1:

1-butanol instead of one-butanol

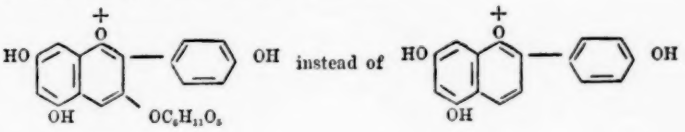
Page 120, Figure 1, second abscissa scale:

0 2 4 6 8 10 instead of 0 2 3 4 5 6

Page 121, Figure 2, lowest number of ordinate scale:

0.01 instead of 0

Page 121, first part of chemical equation:



Page 127, line 23:

curves instead of curve.